A Phase II Clinical Trial of Anti-Tac(Fv)-PE38 (LMB-2) Immunotoxin for CD25 Positive Hairy Cell Leukemia
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Title: A Phase II Clinical Trial of Anti-Tac(Fv)-PE38 (LMB-2) Immunotoxin for CD25 Positive Hairy Cell Leukemia

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Drug Name:	anti-Tac(Fv)-PE38 (LMB-2)
IND Number:	6662
Sponsor:	CTEP
Manufacturer:	Monoclonal Antibody and Recombinant Protein facility, NCI, Frederick, MD
Supplier	NCI

Commercial Agents: none

PRÉCIS

Background.

About 80% of patients with hairy cell leukemia (HCL) have malignant cells that express CD25 (Tac or IL2Ra). Normal resting B- and T-cells do not express CD25. LMB-2 is an anti-CD25 recombinant immunotoxin containing variable domains of MAb anti-Tac and truncated Pseudomonas exotoxin. A phase I trial at NCI found that the MTD of LMB-2 was 40 μg/Kg IV given every other day for 3 doses (QOD x3). The most common adverse events were transient fever, hypoalbuminemia and transaminase elevations. In that trial, 4 of 4 patients with chemoresistant HCL had major responses, including one complete (CR) and 3 partial remissions. The patient with CR entered the trial transfusion dependent and now still has normal hemoglobin and platelet counts over 7 years later. Because HCL is more frequently CD22+ than CD25+ (100 vs 80%), HCL patients were subsequently treated with the anti-CD22 recombinant immunotoxin BL22 and no further HCL patients were treated with LMB-2. BL22 has induced 25 CRs out of 51 evaluable HCL patients. LMB-2 may be useful in patients incompletely responding to BL22, because it may distribute more evenly through extravascular sites of disease. Moreover, BL22 but not LMB-2 has caused hemolytic uremic syndrome (HUS) in 7 patients, 6 with HCL, and several of these patients could benefit by LMB-2. Thus, LMB-2 may be a useful and potentially lifesaving agent in patients who are unable to receive or who have not responded adequately to BL22.

Objectives. The purpose of this study is to determine the activity of anti-Tac(Fv)-PE38 (LMB-2) in patients with CD25-expressing hairy cell leukemia (HCL). The primary endpoint of this trial is response rate.

Eligibility. Patients must have CD25+ HCL cells by flow cytometry, cytopenia or high circulating HCL count, prior treatment with or inability to receive BL22, prior treatment with cladribine, ECOG PS 0-2, at least 18 years old, ALT and AST grade 0-2, albumin grade 0-1, bilirubin ≤ 2.2, creatinine ≤ 1.4 or creatinine clearance ≥ 50, lack of high levels of neutralizing antibodies, lack of anti-CD25 Mab therapy for 12 weeks and other systemic treatment for 4 weeks, no prior treatment with LMB-2, lack of other uncontrolled illness including 2nd malignancy, no HIV or hepatitis C positivity, no coumadin therapy, LVEF $\geq$ 45%, DLCO $\geq$ 55%, and FEV1 $\geq$ 60%.

Design. Patients will receive LMB-2 at 40 ug/Kg QOD x3 at intervals of at least 25 days for up to 6 cycles. Retreatment is permitted in the absence of neutralizing antibodies or progressive disease. Patients in CR may receive 2 consolidation cycles, or 4 consolidation cycles if CR is with minimal residual disease.

Dose level: LMB-2 40 ug/Kg QOD x3

Expected Accrual: 5-10 patients/year, total of 25 evaluable patients

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STATEMENT OF COMPLIANCE

The trial will be carried out in accordance with International Conference on Harmonisation Good Clinical Practice (ICH GCP) and the following:

- United States (US) Code of Federal Regulations (CFR) applicable to clinical studies (45 CFR Part 46, 21 CFR Part 50, 21 CFR Part 56, 21 CFR Part 312, and/or 21 CFR Part 812)

National Institutes of Health (NIH)-funded investigators and clinical trial site staff who are responsible for the conduct, management, or oversight of NIH-funded clinical trials have completed Human Subjects Protection and ICH GCP Training.

The protocol, informed consent form(s), recruitment materials, and all participant materials will be submitted to the Institutional Review Board (IRB) for review and approval. Approval of both the protocol and the consent form must be obtained before any participant is enrolled. Any amendment to the protocol will require review and approval by the IRB before the changes are implemented to the study. In addition, all changes to the consent form will be IRB-approved; an IRB determination will be made regarding whether a new consent needs to be obtained from participants who provided consent, using a previously approved consent form.

1 OBJECTIVES

1.1 PRIMARY

- To determine the response rate of LMB-2 in patients with CD25-positive Hairy Cell Leukemia (HCL).

1.2 SECONDARY

- To describe the response duration.
- To describe how blood levels of LMB-2 (AUC, Cmax) are related to toxicity.
- To describe how the development of neutralizing antibodies affects blood levels of LMB-2 and toxicity.
- To describe how soluble Tac-peptide (sIL2Ra) levels correlate with response to treatment with LMB-2.

2 BACKGROUND

2.1 HAIRY CELL LEUKEMIA (HCL)

Hairy cell leukemia (HCL) is a B-cell malignancy comprising 2% of all leukemias [1, 2], and is highly responsive but not curable with known therapy. Patients present with pancytopenia and splenomegaly, and have malignant cells in the blood, marrow and spleen with eccentric kidney-shaped spongiform nuclei and hair-like cytoplasmic projections. The diagnosis was classically made by tartrate resistant acid phosphatase (TRAP) staining [3, 4]. More recently the diagnosis is most accurately made by fluorescent activated cell sorting (FACS) analysis (or flow cytometry), which demonstrates HCL cells strongly positive for B-cell antigens CD19, CD20, and CD22, and other antigens including CD103 (B-ly7) CD11c, CD25, and CD123 [5-7]. The differential diagnosis, which is important to consider in relapsed and refractory patients, includes splenic lymphoma with villous lymphocytes (SLVL), which may be distinguished from HCL by polar distribution of villous cytoplasmic projections, lack of pancytopenia, and CD25, CD103 and/or CD123 negativity [7-9]. HCL variant (HCLv) is a disorder comprising about 20% of

HCL where the malignant cells may have bilobed nuclei, prominent nucleoli, and clumped malignant cells in the marrow. The HCL cells may be CD25, CD103, CD11c and/or CD123 negative and patients are primarily refractory to purine analogs and other therapies [6, 7, 10-12].

Cladribine as standard therapy for HCL. Cladribine, also called 2-chlorodeoxyadenosine (CdA), is a purine analog inhibitor of ribonucleotide diphosphate reductase and DNA polymerase-a after phosphorylation by deoxycytidine kinase to 2-chloro-dATP [13]. Normally hairy cells are very sensitive because of high deoxycytidine kinase levels and low 5'-nucleotidase activity [14]. Saven et al. reported 91% complete remissions (CRs) and 6% partial responses (PRs) out of 349 evaluable patients, with only 24% CRs relapsing at a median of 30 months [15]. A total of 205 of these patients plus 5 others were followed for at least 7 years, and 95% of 207 evaluable patients achieved a CR lasting 8-172 (median 99) months and 34% of CRs relapsed [16]. The Group C phase II study of cladribine in HCL involved 979 patients treated by local physicians, and of 861 evaluable for response, there were 50% CRs and 37% PRs, although 13% of the PRs might have been CRs if bone marrow biopsy which was missed had been negative [17]. Nevertheless, the relapse free survival of 84% at 4 years was similar to the 79% reported from the Scripps study.

Pentostatin as alternative standard therapy in HCL. Pentostatin (2'-deoxycoformycin, DCF) is a purine analog inhibitor of adenosine deaminase (ADA), an enzyme elevated in acute lymphocyte leukemia cells but low in HCL cells [18]. The largest prospective trial of pentostatin in HCL was in the Intergroup study, where 72% CRs were reported in 154 initially treated plus 87 treated after interferon failure [19, 20]. Relapse-free survival was 85% and 67% at 5 and 10 years. In a retrospective trial of 238 patients including 230 evaluable for response, 66% had CR, 13% had CR not confirmed by bone marrow biopsy, and 17% had PR [21]. No trial has prospectively compared cladribine and pentostatin. Pentostatin is not used as often as cladribine because of the need to dose every other week for several months instead of a single 1 week course, and pentostatin may have more gastrointestinal toxicity.

Prognosis with standard therapy in HCL. Neither of the above-mentioned long term follow-up studies of CdA show a plateau in the disease-free survival curve [15, 16]. Moreover, patients who are in CR after purine analog therapy were found to have PCR-detectable HCL [22, 23]. Although purine analogs are highly effective even in relapsed patients with HCL, several large studies indicate declining CR rates and/or durations with each repeated course [15, 16, 24]. Lack of curability combined with declining efficacy with each retreatment points to eventual loss of ability to effectively treat the disease. Clearly many patients may die of other causes, but those who live long enough may be predicted to progress and eventually die of HCL-related pancytopenia. With over 15 years passing since the introduction of cladribine in the effective treatment of HCL, a significant number of patients with cladribine-resistant disease have accumulated over time, emphasizing the need for development of new approaches.

2.2 SALVAGE THERAPY FOR HCL

Splenectomy and interferon are older treatments for HCL which have a low rate of CR but can provide significant palliative benefit in selected cases [25-28]. Splenectomy can be accomplished safely in most patients by laparoscopy [29, 30]. While normal blood counts temporarily improve, there is increased hairy cell infiltration into the bone marrow, blood and

abdominal lymph nodes [31]. Interferon is sometimes recommended for relapsed HCL, particularly when cytopenias are severe and avoidance of myelotoxicity from purine analogs is a goal [32]. Fludarabine, though known for its excellent efficacy in CLL, is capable of inducing responses in HCL [33, 34]. Rituximab, targeting CD20, has been tested in 4 small trials of HCL patients [35-38], in which a total of 18 (30%) out of 60 patients achieved CR. The 2 trials with the highest CR rates enrolled some patients without cytopenias [35, 38] and in one trial up to 12 doses of rituximab were administered [38]. Thus, rituximab is an effective and well tolerated agent for HCL, at least in a minority of patients, but would not be considered standard therapy for this disease.

2.3 LMB-2 DEVELOPMENT

2.3.1 Anti-Tac

One strategy to enable a monoclonal antibody to kill cells is to use the antibody to block a growth factor receptor. IL-2 is a growth factor for antigen-stimulated T cells and is responsible for T cell clonal expansion after antigen recognition. It is primarily produced by CD4+ T cells. The high-affinity IL-2 receptor (IL-2R) consists of 3 noncovalently associated proteins called Anti-Tac alpha (CD25), beta (CD122), and gamma (CD132), the latter two being members of the type I cytokine receptor family. The CD25 and CD122 chains are involved in cytokine binding, and the CD122 and CD132 chains are involved in signal transduction. CD25, originally called p55 or the Tac antigen (for T cell activation), is a 55-kD polypeptide that normally appears when T cells are activated. It is usually not expressed on resting T-cells, B-cells or monocytes [39]. However, CD122-CD132 (intermediate-affinity IL-2R) is expressed on resting (naïve) T cells and binds IL-2 at 1 x 10^{-9} M concentration. When T cells are stimulated and produce CD25 chains the affinity of IL-2 increases to 1 x 10^{-11} M. Chronic T cell stimulation can lead to the shedding of a 45-kD piece of CD25 into the blood. The serum soluble Tac (sCD25) level can then be followed as a marker of strong antigenic stimulation such as in rejection of a transplanted organ [40].

Anti-Tac is a monoclonal antibody which binds to IL2Rα (CD25) with high affinity blocking the interaction of IL2 with the IL2R [41-45]. Unmodified humanized anti-Tac monoclonal antibody (daclizumab, NSC-277524, IND#2091) was tested in 19 ATL patients (NIH 83-C-0023) to determine if it would block IL2 from stimulating the malignant cells [46, 47]. There was no significant toxicity. Six patients had remissions including 4 partial and 2 complete remissions lasting from one to more than 43 months after onset of therapy. Five of the responding patients relapsed following therapy. Relapses were not due to loss of CD25 expression by the ATL cells, but rather to loss of dependence of the ATL cells on IL2 for their proliferation. Thus, CD25 remains a target for immunotherapy.

2.3.2 PE38

The full-length 613 amino acid PE protein contains three functional domains which are necessary for cellular intoxication [48, 49]. Domain Ia (amino acids 1-252) is the binding domain, domain II (amino acids 253-364) is for translocating the toxin to the cytosol and domain III (amino acids 400-613) contains the ADP ribosylating enzyme which inactivates elongation factor 2 (EF-2) in the cytosol and results in cell death. The function of domain Ib (amino acids

365-399) is unknown. A current model of how PE kills cells includes the following steps: 1) The C-terminal residue (lysine-613) is removed by a carboxypeptidase in the plasma or culture medium [50]. 2) Domain Ia binds to the a2 macroglobulin receptor, present on animal cells [51]. 3) After internalization at low pH, domain II is proteolytically cleaved between amino acids 279 and 280 by furin [52-54]. 4) The disulfide bond between cysteines 265 and 287 which joins the two fragments is reduced, producing an N-terminal fragment of 28 kDa and a C-terminal fragment of 37 kDa. 5) Amino acids 609-612 (REDL) bind to an intracellular sorting receptor which transports the 37 kDa carboxy terminal fragment from the transreticular Golgi apparatus to the endoplasmic reticulum [55, 56]. 6) Amino acids 280-313 mediate translocation of the toxin to the cytosol [57, 58]. 7) The ADP ribosylating enzyme composed of amino acids 400-602 inactivates EF-2 [59]. PE40 is a truncated derivative of PE which is missing the binding domain and hence will not bind specifically to cells unless attached to an antibody or growth factor [48, 60]. PE38 is a truncated version of PE40 which is missing amino acids 365-380. While PE40 and PE38 have similar activities [61, 62], we have preferred to use PE38 because it is slightly smaller and is missing a disulfide bond that impairs refolding the protein.

2.3.3 The Recombinant Immunotoxin

To target cells with a single-chain protein containing the antigen binding domains of anti-Tac, the variable domains of the antibody (V_H and V_L) were fused together with the peptide linker $(G_4S)_3$ and the resulting Fv fragment of anti-Tac was fused to PE40 [63]. Anti-Tac(Fv)-PE40 was extremely cytotoxic with an IC_{50} of 0.15 ng/ml toward HUT-102 cells [63] and 0.05-0.1 ng/ml toward activated human T-cells [62, 64]. To determine if malignant cells in patients have enough receptors and metabolize the toxin effectively enough to be sensitive to anti-Tac(Fv)-PE40, we tested ATL cells from the blood of 38 patients and from the lymph nodes of 5 patients. All samples were sensitive to anti-Tac(Fv)-PE40, with IC_{50}'s of 0.03-16 ng/ml [62, 65-67]. Anti-Tac(Fv)-PE40 was shortened slightly by removing amino acids 365-380, resulting in anti-Tac(Fv)-PE38 (LMB-2). The cytotoxic activity of anti-Tac(Fv)-PE38 (LMB-2) appears identical to that of anti-Tac(Fv)-PE40 toward cell lines and fresh ATL samples [62]. Importantly, it was shown that these recombinant anti-CD25-immunotoxins are still cytotoxic in the presence of soluble CD25 concentrations as high as those expected in ATL patients [67]. This suggested that even in ATL patients, whose sera have the highest levels of soluble CD25, anti-Tac(Fv)-PE38 (LMB-2) should not be prevented by soluble CD25 from reaching the malignant cells.

2.3.4 Preclinical studies of LMB-2 *in vivo*

A mouse model of a human CD25 positive malignancy was produced by the subcutaneous injection in nude mice of ATAC-4 cells [68]. These cells are A431 epidermoid carcinoma cells that have been transfected with the gene encoding CD25, and contain 2×10^5 CD25 sites/cell [62]. Mice began treatment with LMB-2 four days after ATAC-4 cell injection, when subcutaneous tumors became established (32-86 mm^3). Ninety-100% tumor regressions were observed in 2 of 5 mice receiving 30 µg/Kg i.v. QD X 3, and in 5 of 5 mice receiving 60 µg/Kg i.v. QD X 3. These doses were respectively 5 and 10% of the mouse LD_{50} [68]. When administered to mice every other day, complete tumor regressions could be obtained in 5 of 5 mice receiving 100 µg/Kg i.v. days 4, 6 and 8. The LD_{10} and LD_{50} are 200 and 257 µg/Kg every other day for 3 doses. The cause of death on necropsy of the mice was liver damage.

Cynomolgus monkeys were used to determine the safety and pharmacokinetics of LMB-2, since anti-Tac reacts with primate but not murine CD25. In a GLP pharmacokinetic study, the elimination of LMB-2 from the serum followed biphasic kinetics, with a $T_{1/2}\alpha$ of 45 minutes and a $T_{1/2}\beta$ of 127 minutes. In a GLP toxicology study, 4 Cynomolgus monkeys received 20 µg/Kg days 1, 3 and 5 with no significant toxicity. Four monkeys were then given 300 µg/Kg days 1, 3 and 5 and experienced dose-limiting toxicity with anorexia and 2 to 4-fold transaminase elevations. One of two monkeys (male) autopsied on day 7 in this high-dose group had hepatomegaly, mild diffuse hepatocyte vacuolation, testicular and epididymal degeneration, and leukocytosis and vascular inflammation of the skin. The other monkey (female) had a ~0.5 X 0.8 mm area of myocardial degeneration. Of the four high-dose monkeys, the one with hepatocyte vacuolation had the mildest (up to 2-fold) transaminase elevations. The two remaining high-dose monkeys were sacrificed on day 51. One of these (male) was found to have a ~0.4 X 0.4 mm area of myocardial degeneration and testicular and epididymal degeneration. The other (female) was found to have a myocardial parasite. No monkeys had detectable CPK-MB positivity or LDH I/II isoenzyme ratio greater than one at any time point. After the toxicology study was completed, it was reported by a group at Parke-Davis Research Institute that untreated wild-caught Cynomolgus monkeys have high rates of various pathologic lesions. In particular, of 62 male and 62 female animals, 45.2% of males and 48.4% of females had cardiac lesions, 58.1% of males and 54.8% of females had hepatic lesions, 31.4% had skin lesions and 13% of males had immature testicular and accessory sex organ histology [69]. Unpublished data from these investigators indicated that 6.4% of the males and 14.5% of the females had myocardial degeneration and/or necrosis, 8.1% of the males and 3.2% of the females had hepatic necrosis, 4.8% of males and 1.6% of females had hepatic vacuolation, 6.4% of males and females had mixed cell infiltrates in skin, 1.6% of males and 3.2% of females had inflammation in the skin and 12.9% of males had immature testes.

2.3.5 Phase I trial of LMB-2 (NIH #96-C-0064)

LMB-2 induced responses in patients with CD25+ chemotherapy-resistant hematologic malignancies, including 4 with hairy cell leukemia (1 CR, 3 PR) and one PR each with adult T-cell leukemia (ATL), Hodgkin's disease (HD), chronic lymphocytic leukemia (CLL), and cutaneous T-cell lymphoma (CTCL) [70, 71]. The HCL patient with CR had a Hgb as low as 3.8 prior to transfusions and continues to have a normal Hgb more than 7 years later. The 3 HCL patients with PR had limited retreatment due to pneumonia, DLT, and neutralizing antibodies. The published Phase I results included 35 patients (age range 24-79), 11 with HD, 6 with B-cell lymphoma, 8 with CLL, 4 with HCL, 3 with PTCL, 1 with CTCL, and 2 with ATL. Of these 35 patients, 22 received 1 cycle only, 8 received 2 cycles, 2 received 3 cycles, and 1 each received 4, 5, and 6 cycles. Twenty patients developed anti-PE38 neutralizing antibodies and 9 of those patients also developed HAMA anti-bodies. Twenty-nine of the 35 patients received a starting dose of ≥ 10µg/Kg QOD x 3. Data discussed below includes 4 patients not in the phase I report, who had ATL (#36), HD (#37), CTCL ((#38) and NHL (#39). Patient #36 received 50 ug/Kg QOD x3 and patients #37-39 received 40 ug/Kg QOD x3. Of these 4 patients, patients #38-39 each received a 2nd cycle and patient #38 developed neutralizing antibodies.

LMB-2 related toxicity with at least one cycle of LMB-2 was seen at ≥ 10µg/Kg QOD x 3 and was reversible. Please see Table 1 below for a list of toxicities. The common toxicity criteria (CTC) of the NCI were used to grade toxicity. Dose Limiting Toxicity (DLT) was defined as at least grade 3 toxicity (attribute possible, probable, or definite), but the following exceptions were not considered to be dose-limiting: (1) transaminases elevations of 5-20 times normal, (2) bilirubin 1.5-2.2 times normal, (3) fever that was well tolerated and did not result in an interruption in therapy, (4) hematologic toxicity in leukemic patients, (5) grade 3 hematologic toxicity in nonleukemic patients, and (6) abnormal coagulation profiles in patients who were receiving anticoagulant therapy or who had preexisting coagulation abnormalities.

DLT was observed in two of three patients at the 63 µg/Kg QOD x 3 dose level. The first patient (with HD) had asymptomatic grade 4 AST and grade 3 ALT elevations. The second patient (with HCL) developed grade 3 diarrhea, grade 2 fever, nausea, and vomiting, and grade 4 cardiomyopathy on day 5. The patient's cardiac function returned to normal by day 7. FACS of peripheral blood on day 9 indicated a large number of dead tumor cells. We concluded that the cardiomyopathy was either cytokine mediated or due to direct LMB-2 toxicity on the heart. The 50 µg/Kg dose level was dose limiting in 1 of 6 patients (patient #27 with PTCL) due to an allergic reaction.

The most common toxicities were transient fever and transaminase (ALT, AST) elevations, usually grade 1 or 2. Grade 3 transaminase elevations were not associated with impaired liver function as assessed by PT, bilirubin, and fibrinogen levels. Grade 1 nausea and vomiting could not be correlated with the degree of transaminase elevations. The transaminase elevations were never observed to increase after day 8 of each cycle in any patient. In all patients with grade 3-4 elevations, the AST and ALT levels resolved to ≤40 U/L.

Fever (22/29 patients) typically occurred within hours of the first dose and did not recur after the second and third doses. Often it recurred on subsequent cycles after the first dose. Eighteen of 22 patients with normal pretreatment albumin levels (≥3.7g/dL) experienced hypoalbuminemia, 11 patients with grade 1 and 7 with grade 2. Most of these patients did not develop significant weight gain and none had symptomatic pulmonary edema. Reversible grade 1 drug-related renal toxicity was observed in 3 patients.

For pharmacokinetic studies, dilutions of patient plasma were tested in cytotoxicity assays against a cell line (SP2/Tac) and compared to a cytotoxicity standard curve created with known amounts of LMB-2. In 12 patients treated at the MTD (40 µg/kg), peak plasma levels after cycle 1 were 340-1040 (median 560) ng/ml, the AUCs were 48-257 (median 123) ug-min/ml, the half-lives were 185-322 (median 216) min, and the clearances were 14-79 (median 25) ml/min). The median values for the 4 patients with HD were 620 ng/ml, AUC 155 ug-min/ml, half-life 193 min, and clearance 19 ml/min.

Patient #36 with ATL was treated at 50 µg/Kg QOD x3 and experienced a reversible but dose-limiting syndrome similar to patient #30, with 3rd spacing and muscle edema causing grade IV CK elevation and hypoventilation leading to supraventricular tachycardia and respiratory failure. Associated toxicity included grade III hypoalbuminemia, grade II ALT, grade III AST, grade II acidosis, grade I fever, grade I GGTP, grade II lipase, grade I thrombocytopenia grade III

hypotension and grade II weight gain. This patient had a muscle biopsy which ruled out necrosis or inflammation, indicating that the CK elevation was from passive muscle fiber edema and stretching. Due to this DLT event (2 out of 6 patients at this level now had DLT), the dose level was reduced to 40 μg/Kg QOD x3.

In phase II testing, LMB-2 has been administered to 5 CLL patients without DLT at 40 ug/Kg QOD. Patient CL03 had 6 cycles and a PR. Patient CL04 had a good response in lymph nodes but not in the peripheral blood after 2 cycles. He had grade II weight gain and CPK elevation with cycle 1, but much less toxicity with cycle 2. He did have a pneumonia after cycle 2 and on day 27 was diagnosed with bilateral occipital infarcts related to a documented patent foramen ovale. On a phase II CTCL protocol, 1 patient was enrolled at 40 ug/kg QOD x3 who had transient grade IV CPK elevation as his only DLT, so the dose level was lowered for future CTCL patients to 30 ug/Kg QOD x3.

Several patients treated with LMB-2 have developed completely reversible allergic reactions other than rash, associated with immunogenicity to LMB-2. Immunogenicity to the toxin domains of similar immunotoxins BL22, HA22 and SS1P is not associated with allergic reactions other than rash, suggesting relationship to the targeting domain, anti-Tac(Fv). Grade 3-4 allergic reactions observed during phase I testing included grade 3 bronchospasm during the 1st dose of cycle 2 in a patient with HD, and grade 4 anaphylaxis during the 1st dose of cycle 1 in a patient with PTCL (73). The patient with anayphylaxis during cycle 1 of LMB-2 had prior humanized anti-Tac Mab (Zenapax, daclizumab) treatment and was in retrospect found to have human anti-human IgG antibody (HAHA), suggesting that the idiotype of LMB-2, which is also present in humanized anti-Tac Mab, plays a role in both immunogenicity and allergic reactions. Of 9 CTCL patients who have undergone phase 1 or 2 testing, one had an allergic reaction during cydle 2, associated with an unusual feeling in the throat. Of 11 HCL patients who have undergone phase 1-2 testing, 2 had syncopal episodes during retreatment, one of whom noted an identical episode with rituximab which was subsequently prevented with steroids. The level of neutralizing antibodies prior to the cycle causing allergic reactions was either low or undetectable and did not correlate with allergic reaction. To prevent these allergic reactions, CTCL patients will receive steroid prophylaxis with a single dose of dexamethasone 20 mg i.v. 2 hours prior to the first dose of each retreatment cycle of LMB-2.

In summary, LMB-2 is well tolerated at the MTD except for 1 patient with CTCL. The most common toxicity, transaminase elevation, was reversible and never associated with any other evidence of hepatic dysfunction. All other toxicities at the MTD were also transient, resolving within a few days to weeks after treatment.

Table 1

LMB-2-related cycle-1 toxicities for patients treated at ≥10μg/Kg QOD x 3 (phase I patients 7-39)

Toxicity	Dose Level: Total patients (total Grade 3 or 4)					
	10	20	30	40	50	63
Total treated	3	3	5	12	7	3
Transaminases	2	2	5(1)	11(3)	7(3)	3(2)
Fever	0	2	5	10	6(1)	3(1)

Myalgia	2	2	1	7	1	1
Alkaline phosphatase	0	0	0	3	0	3
Thrombocytopenia	0	1	0	3(1)	2	1
Hypoalbuminemia	1	1	4	7	4(1)	2
Hypotension	0	0	0	4	4(1)	2
Nausea/Vomiting	0	0	0	7	2	3
Diarrhea	0	0	0	1	1	1(1)
Pericardial effusion	0	1	1	2	0	2
Weight Gain	1	0	1	5	2	2
Allergy	0	0	0	2	2(1)	0
Increased Creatinine	0	0	0	3	2	0
Proteinuria	0	0	0	2	1	0
Cardiomyopathy	0	0	0	0	0	1(1)
Total Evaluated	3	3	5	12	7	3

Table 2

Phase I LMB-2-related toxicities for the 12 patients treated at the MTD (40 μg/Kg)

Toxicity	Grade			Total # of patients
	1	2	3	
Transaminases	6	2	3	11
Fever	3	7		10
Myalgia	5	2		7
Alkaline phosphatase	2	1		3
Thrombocytopenia	2	1		3
Hypoalbuminemia	2	5		7
Hypotension		4		4
Nausea	5	2		5
Vomiting	1	1		2
Creatinine/Proteinuria	3			3
Pericardial effusion	2			2
Weight Gain	3	2		5
Diarrhea		1		1

2.3.6 LMB2 Test Dose

All patients on the Phase I trial received a 10 μg test dose before the first dose of LMB2 on day 1 of each cycle. The test dose was administered 30 minutes prior to the LMB2 treatment dose which was 30 minutes after pre-medication. There was no correlation between patient tolerance of test doses and LMB2 treatment. Notably, no patients were taken off treatment due to test dose reactions. Furthermore, none of the patients who had allergic toxicity had sustained reactions to the test dose. Due to this lack of utility, test doses will not be administered on this trial.

2.3.7 Efficacy of anti-CD22 recombinant immunotoxin in HCL

Fifty-three patients with hairy cell leukemia (HCL) previously treated with CdA were treated with BL22, 31 in phase I testing, 2 by special exemption, and 20 in phase II testing. The complete remission rate in phase I was 19 (61%) out of 31 patients, and 6 (19%) had partial response (PRs) [72, 73]. The median (range) duration of CR was 36 (5-73) months and 7 (37%) patients are still in CR at a median of 51 (range 35-73) months. Of 30 with cytopenias, 22 (73%) achieved hematologic remission (HR), with neutrophils, platelets, and hemoglobin at least 1500, 100,000 and 11. Nine (41%) are still in HR at a median of 50 (35-73) months. Of 19 evaluable HCL patients on phase II, 8 (42%) achieved CR, 3 (16%) PR, and 3 (16%) who are not yet in CR are still under retreatment. Only 2 phase II patients have relapsed from CR, both of whom had no consolidation cycles of BL22. BL22 is safe at 40 ug/Kg QOD x3 for cycle 1, with no BL22-related SAEs observed in 12 of 12 patients in phase I, 2 of 2 patients on special exemption, and 20 of 20 patients on phase II. However, with retreatment at 30-50 ug/Kg QOD x3, 6 HCL patients have had a reversible hemolytic uremic syndrome (HUS), during cycles 2 or 3. The first 5 HUS cases in HCL were treated with plasma exchange, but the 6th patient was not and still had complete recovery. HUS has not been observed with LMB-2 or any other PE38-containing toxin besides BL22, indicating that the mechanism of HUS is mediated by both the toxin and CD22-binding domains. HUS due to BL22 has also been observed in 1 patient each with non-Hodgkin's lymphoma and CLL, indicating that the syndrome is probably not dependent on the HCL phenotype. High levels of neutralizing antibodies were observed in 1 out of 31 phase I HCL patients after cycle 1, but with retreatment of up to 30 cycles over nearly 5 years in some patients, 11 (35%) made high levels of neutralizing antibodies overall. Neutralizing antibodies to BL22 include antibodies to the toxin, and such patients would not be eligible for LMB-2. However, only 1 out the 6 HCL patients with HUS made neutralizing antibodies to BL22, and thus the other 5 patients, who are ineligible for BL22 because of prior HUS, would be eligible for LMB-2.

2.4 RATIONALE OF STUDY DESIGN

2.4.1 CD25 expression in HCL. CD25 is expressed on about 75-80% of HCL samples [6]. LMB2 is a recombinant immunotoxin that targets CD25.

2.4.2 Response of HCL to LMB-2. In the phase I dose-escalation trial of LMB2, there were 1 CR and 3 PRs out of 4 HCL patients [70, 71]. Several of these responders could not be effectively retreated either because of neutralizing antibodies or disease-related infections.

2.4.3 Need for LMB-2 in HCL. BL22 is clearly the preferred agent for HCL due to 20-25% of patients who have CD25 negative HCL cells. Thus HCL patients eligible for LMB-2 would have already received BL22 and not be eligible or appropriate for further BL22. Such patients include 2 groups:

2.4.3.1 Patients who had HUS from BL22. Currently 8 CD25+ HCL patients have had HUS from BL22. Several of these might be appropriate for further BL22 at a lower dose level, but since LMB-2 has never caused HUS, the dose they could receive of LMB-2 would be significantly higher and hence their chance of response much better.

2.4.3.2 Patients who did not completely respond to BL22 despite multiple cycles. A possible reason for lack of CR could be that since CD22 is expressed at such high levels in HCL (up to 100,000 sites/cell), patients with significant tumor burden could have very limited distribution of BL22 to all sites of tumor. HCL, while being very sensitive to LMB-2 [74], express only about 1300-17,000 sites/cell and the half-life is slightly longer than BL22. A total of 6 out of 31 patients on phase I and 5 out of 18 on phase II might have benefited from LMB-2 due to improved distribution. Several of these patients are still alive without neutralizing antibodies and might benefit by LMB-2.

2.5 DOSE DETERMINATION

The MTD established in the phase I trial of LMB-2 in 12 patients was 40 ug/Kg QOD x3. In phase II testing, Four out of 5 patients with CLL tolerated this dose level without DLT, and one patient with CTCL had grade IV CPK elevation without other evidence of DLT. This led to a lowering of the phase II dose level in the CTCL trial to 30 ug/Kg QOD x3. Because HCL is more like CLL in having an intravascular component, we plan to use the 40 ug/Kg QOD x3 dose level. LMB-2 will be given every 4 weeks for up to 6 cycles.

3 ELIGIBILITY ASSESSMENT AND ENROLLMENT

3.1 ELIGIBILITY CRITERIA

3.1.1 Inclusion Criteria

3.1.1.1 Histopathological evidence of CD25+ HCL confirmed by the NIH pathology department. This will require a monoclonal population of peripheral malignant lymphocytes that are CD25 positive by fluorescence activated cell sorting (FACS) with anti-CD25 antibody. Positive expression in a FACS assay is defined as more than 2 times the mean fluorescence intensity (MFI) of the control antibody by FACS. HCLv (HCL variant) is usually CD25 negative, and eligibility would require CD25+ HCLv.

3.1.1.2 At least one of the following indications for treatment: neutropenia (ANC <1000 cells/ul), anemia (Hgb <10g/dL), thrombocytopenia (Plt <100,000/ul), an absolute lymphocyte count of >20,000 cells/ul or symptomatic splenomegaly.

3.1.1.3 Previous treatment with or inability to receive BL22 or HA22 recombinant immunotoxin. Patients must have had at least 2 prior systemic therapies, including 2 courses of a purine nucleoside analog (PNA), or 1 course of either rituximab or BRAF inhibitor following a single prior course of PNA.

3.1.1.4 ECOG performance status of 0 – 2.

3.1.1.5 At least 18 years old.

3.1.1.6 Understand and give informed consent.

3.1.1.7 A negative pregnancy test in female patients of childbearing potential. Women must not be breast-feeding (see section **5.1.1.1**).

3.1.1.8 ALT and AST ≤5-times the upper limits of normal. Albumin ≥3.0 gm/dL. Total bilirubin ≤2.2 mg/dL.

3.1.1.9 Creatinine ≤1.4 mg/dL or creatinine clearance ≥50 ml/min.

3.1.1.10 Serum that neutralizes ≤75% of the activity of 1 μg/mL of LMB-2 using a bioassay.

3.1.1.11 No systemic cytotoxic chemotherapy within 4 weeks of enrollment or systemic steroids (except stable doses of Prednisone ≤20 mg/day, or up to 4 doses of steroid given for non-therapy reasons) within 4 weeks of enrollment.

3.1.1.12 No anti-CD25 monoclonal antibody therapy within 12 weeks of enrollment.

3.1.1.13 No prior treatment with LMB-2.

3.1.1.14 Patients may not be receiving any other investigational agents.

3.1.1.15 Patients should not have uncontrolled intercurrent illness including, but not limited to, symptomatic congestive heart failure, unstable angina pectoris, cardiac arrhythmia, or psychiatric illness/social situations that would limit compliance with study requirements.

3.1.2 Exclusion Criteria

3.1.2.1 Patients who have HIV or hepatitis C. Patients would not be excluded for hepatitis B surface antigen positivity if on Lamivudine.

3.1.2.2 Patients receiving coumadin.

3.1.2.3 Patients with a left ventricular ejection fraction of < 45%.

3.1.2.4 Patients with a DLCO <55% of normal or an FEV1 <60% of normal, based on either NIH or USA normal ranges.

3.1.2.5 Patients who have an active 2nd malignancy requiring systemic treatment.

3.2 RESEARCH ELIGIBILITY EVALUATION

- A blood sample will be submitted to the NIH pathology department for analysis of CD25 expression (an eligibility criterion) by FACS analysis. This analysis will be done in the Laboratory of Pathology, NIH (Anatomic Pathology Dept., CC), a College of American Pathologists (CAP) and CLIA-approved laboratory, by standardized diagnostic techniques.
- Complete history and physical examination with documentation of measurable disease (lymph nodes, spleen, liver), stage, and performance status within 2 weeks before starting LMB-2. Physical examination should record the diameter, in two planes, of the largest palpable nodes in each of the following sites: cervical, axillary, supraclavicular, inguinal, and femoral. Physical examination should record liver and spleen size as determined by measurement below the respective costal margin.

- ECG and CXR within 3 days before starting LMB-2.
- CT scan of the chest, abdomen, and pelvis, or abdominal MRI, within 4 weeks before starting LMB-2.
- Serum anti-LMB-2 antibody assay, HIV, Hepatitis B surface antigen, and hepatitis C screen within 2 months before starting LMB-2.
- Laboratory evaluation within 2 weeks before starting LMB-2 will include CBC/differential, acute care panel (electrolytes, glucose, BUN, creatinine), hepatic panel (AST, ALT, Alkaline phosphatase, total and direct bilirubin), albumin, LDH, PT, Beta 2 microglobulin (B2M), serum protein electrophoresis (SPEP), urinalysis, and a 24 urine creatinine clearance and total protein. A CBC/differential, acute care panel, hepatic panel, and albumin must be done within 3 days of starting cycle 1.
- Urine or serum pregnancy test within 72 hours before starting LMB-2 in women with childbearing potential.
- Bone marrow biopsies and echocardiogram with left ventricular ejection fraction calculation, and stress test, will be done on all patients within 2 months before starting LMB-2.

3.3 Inclusion of Women and Minorities

Inclusion of Women and Minorities. No eligible women and minorities will be excluded from the protocol. There is no basis for exclusion of otherwise eligible patients on the basis of age, gender or race.

3.4 Recruitment Strategies

This protocol may be abstracted into a plain language announcement posted on NIH websites and on NIH social media platforms.

3.5 Participant Registration and Status Update Procedures

Registration and status updates (e.g., when a participant is taken off protocol therapy and when a participant is taken off-study) will take place per CCR SOP ADCR-2, CCR Participant Registration & Status Updates found at:
https://ccrod.cancer.gov/confluence/pages/viewpage.action?pageId=73203825.

3.5.1 Screen Failures

Screen failures are defined as participants who consent to participate in the clinical trial but are not subsequently assigned to the study intervention or entered in the study. A minimal set of screen failure information is required to ensure transparent reporting of screen failure participants, to meet the Consolidated Standards of Reporting Trials (CONSORT) publishing requirements and to respond to queries from regulatory authorities. Minimal information includes demography, screen failure details, and eligibility criteria.

4 STUDY IMPLEMENTATION

4.1 Study Design

This is a Phase II study to test the response of patients with HCL to LMB-2. LMB-2 will be given every 28 days (minimum of 25 days) for up to 6 cycles. No patient whose serum neutralizes > 75% of the activity of 1 µg/mL of LMB-2 will be retreated. In patients who

achieve a CR with minimal residual disease (MRD), defined as positive immunohistochemistry of bone marrow biopsy or flow cytometry of blood, no more than 4 additional cycles of LMB-2 will be administered. Patients with CR without MRD may receive 2 additional cycles. Patients who relapse after >2 months of a CR or PR are eligible for retreatment with LMB-2 alone on the same schedule if they still meet initial eligibility criteria and were not removed from study due to toxicity, provided that the study is still open and the LMB-2 supply permitting. Stopping rules in this case will be the same as those during initial LMB-2 administration. Patients will be treated with LMB-2 as described below. Response and duration of response will be determined as described in section **13**.

4.2 DRUG ADMINISTRATION

LMB-2 Infusion: 40 µg/Kg will be infused through a peripheral I.V. or central line in 50 ml of 0.9% NaCl and 0.2% albumin via a PAB container over 30 minutes every other day for 3 doses (QOD x 3). Additional IV fluid will be given as described below. The first cycle will be administered on the Oncology Inpatient Unit. If treatment is tolerated well, then subsequent doses can be administered on an outpatient basis.
Premedication: Patients will be medicated with 25 mg hydroxyzine and 150 mg ranitidine orally 1 hour prior to and 8 hours after each dose. Acetaminophen 650 mg P.O. will be given every 6 hours for 4 doses starting 1 hour prior to each LMB-2 dose. Dexamethasone 20 mg i.v. will be given 2 hours prior the 1st dose of each retreatment cycle. While the times of concomitant medications are recommended, the actual time of administration should be documented particularly when they differ. Emergency medications such as epinephrine and diphenhydramine should be available in the area where the patients will receive the LMB-2 infusion for treatment of an allergic reaction. Emergency equipment including oxygen should be available in the patient's room.
I.V. Fluid: Patients will receive fluid prophylaxis, consisting of 1000 ml of D5/0.45% NaCl over 2-4 hours prior to LMB-2 and another 1000 ml of D5/0.45% NaCl over 2-4 hours after the LMB-2 infusion is completed. (This is approximately the same amount of IVF given to the last 4 patients on the phase I protocol. However, it is in a more simple administration format). Once the first dose of each cycle is given, patients will receive normal saline, at least 40 cc/hr until day 5-8 when not receiving another type of I.V. fluid.

4.2.1 Vital signs of inpatients will be obtained at the beginning of infusion, at 15 minutes, & at the end of infusion, then every 60 minutes for 2 hours, then as per unit routine. Daily orthostatic blood pressure, weights, I/Os, and physical exam will be done. Daily orthostatic blood pressure will be measured after having the patient in supine and standing position for at least 2 minutes. If standing SBP decreases from supine SBP by > 20 mmHg, then another standing blood pressure measurement will be done at least 3 minutes after the first. Please see section **4.8** for management of hypotension. Vital signs of outpatients will be obtained at the beginning of infusion, at 15 minutes, & at the end of infusion, then every 60 minutes for 2 hours. Patients will be monitored for peripheral edema and orthostatic hypotension at least daily. Daily weights will also be recorded. Please see section **4.8** for management of hypotension. Determination of DLT

DLT is defined as LMB-2-related grade III-V toxicity, with exceptions below.

4.2.2 Definition of VLS

Definition of Vascular Leak Syndrome (as stipulated by CTEP to be utilized until December 31, 2010): As specified by CTC 3.0, grade II VLS includes symptoms of fluid retention; this protocol further defines that if weight gain is the only feature of VLS in a patient, it should be grade I-II to be classified as grade II VLS. If a patient requires more than an hour's worth of hydration at 20 ml/Kg/hour for hypotension, then the patient will be considered to have grade III hypotension. Grade III hypotension in temporal association with VLS will be considered also as grade III VLS. VLS resulting in respiratory compromise is considered grade III according to CTC 3.0. Respiratory compromise is defined as symptomatic pulmonary edema requiring oxygen or > 10% decrease in oxygen saturation.

Definition of Capillary Leak Syndrome (as stipulated by CTEP) to be utilized after January 1, 2011: As specified by CTCAE 4.0, grade II CLS is defined as symptomatic; medical intervention is indicated; this protocol further defines that if weight gain is the only feature of CLS in a patient, it will be considered a grade II CLS. If a patient requires more than an hour's worth of hydration of 20 ml/Kg/hour for hypotension, then the patient will be considered to have grade III hypotension. Grade III hypotension in temporal association with CLS will be consider also a grade III CLS. Respiratory compromise in the setting of CLS defined as symptomatic pulmonary edema requiring oxygen or > 10% decrease in oxygen saturation will be consider a grade III CLS. Grade III hypotension or grade III CLS is dose limiting.

4.2.3 Treatment Modifications for Toxicities on Current Cycle

Treatment should not be given on Day 3 or 5 until the laboratory values are reviewed for creatinine, AST, ALT, and GGT to determine if treatment modifications are necessary.
Grades I and II Allergic Reaction/hypersensitivity (including drug fever):

- Drug fever only: give acetaminophen every 4-6 hours until resolved (see section **4.8**).
- Rash (under Allergic reaction in CTC 3.0 to be utilized until December 31, 2010 and under Skin and Subcutaneous Tissue Disorder in CTCAE 4.0 to be utilized after January 1, 2011): follow guidelines in section **4.8** and hold treatment (up to 3 days) until symptoms resolve.

Grade II Creatinine: Drug will be held (up to 3 days) until Creatinine is ≤ Grade I.
Grade III Allergic Reaction or Grade II Allergic Reaction with asymptomatic bronchospasm or urticaria: Stop treatment. Off treatment (DLT).
Other grade III toxicities except for AST, ALT, GGT, and hematologic: Stop treatment. Cycle ends (DLT).
Grade IV toxicities except for grade 4 hematologic toxicity lasting ≤ 5 days: Stop treatment. Off treatment (DLT).
Grade IV hematologic toxicity lasting > 5 days except for alopecia: Off treatment (DTL).
Grade IV CPK associated with any other DLT or not resolving to < grade II within 2 weeks: Off treatment (DLT).

4.2.4 Treatment Modifications for Toxicities on Previous Cycle

Retreatment:

- All drug-related non-preexisting toxicities should recover to ≤ Grade I prior to retreatment except AST, ALT, GGT, must recover to ≤ Grade II.

- Patients who entered the study with ≥ Grade II baseline (abnormal) hematological values should have recovered to baseline values prior to treatment.

Dose reduction if at least 1 of the following occurred:

- Grade III toxicities except for AST, ALT, GGT, fever, and hematologic.
- 2nd or 3rd dose held >3 days (due to toxicity)
- Grade II creatinine

4.2.5 Dose Reductions:

- The 1st dose reduction is to 30 μg/Kg QOD x 3.
- The 2nd dose reduction is to 20 μg/Kg QOD x 3.
- If a 3rd dose reduction is required, patient is off treatment.
- There will be no dose re-escalation.

4.3 PHARMACOKINETIC STUDIES

4.3.1 Analysis of LMB-2 in the plasma

Blood samples will be drawn by the patient care unit registered nurse at the times outlined below. Tubes must be labeled with the patient's name, medical record number, date of birth, date, time drawn, and time related to LMB-2 dose. Samples drawn from central venous catheters may be used as long as it is flushed with 5ml of 0.9% sodium chloride prior to obtaining the 2 minute post-infusion sample. Samples of 2 ml of blood will be drawn in a 6 ml sodium heparin tube (green top). Samples of 2 ml blood in serum separator tubes will be drawn with each green top tube to allow ELISA assays to be run on stored serum samples. Tubes of blood collected at the clinical research center (including both inpatient and outpatient units) should be stored upright in the "Kreitman" container in the designated refrigerator for this purpose. These samples will be collected daily Monday through Friday and taken to our lab for analysis.

4.3.1.1 Days 1 and 5

Inpatients: Pre-dose, 2 minutes after the end of the LMB-2 30-minute infusion, then at 1, 2, 4, 8, and 24 hours.

Outpatients: Pre-dose, 2 minutes after the end of the LMB-2 30-minute infusion, then at 1, 2, and 24 hours.

4.3.1.2 Day 3

Pre-dose, 2 minutes after the end of the LMB-2 30-minute infusion, then at 24 hours.

The acceptable error for PK time points is +/- 2 minutes for 2 minute sample; +/- 15 minutes for other samples. PK time points are recommended, and if the actual time point differs, the actual time of sample collection should be clearly written on the tube.

4.3.2 Analysis of neutralizing antibodies & soluble Tac

At least 2 ml of blood will be drawn in a Serum Separator Tube (SST) prior to starting each cycle and between days 17-25 of each cycle. Tubes must be labeled with the patient's name, medical record number, date of birth, date, and time drawn. Tubes of blood collected at the clinical research center should be stored upright in the "Kreitman" container in a designated refrigerator. These samples will be collected daily Monday through Friday and taken to our lab for analysis. Tubes of blood collected outside of the clinical center should be sent by Fed Ex to: David Waters, Ph.D. — NIH/SS1 Leidos Biomedical Research, Inc., Building 535, Rm. 428A — Frederick, MD 21702 — Phone: 301-846-5831.
The presence of antibodies to LMB-2 is determined by a bioassay performed in a CLIA-certified lab in Frederick. In a 96-well U-bottom plate, serum from patients, or 0.2% human serum albumin in PBS (HSA-PBS), is mixed with different concentrations of LMB-2 which are diluted in HSA-PBS. After mixing with serum, the serum-toxin mixtures each contain 90% serum and either 0, 40, 200 or 1000 ng/ml of LMB-2. These mixtures are incubated at 37C for 15 minutes and diluted into cell culture media (DMEM+10% FBS) in a U-bottom 96-well plate so that the final toxin concentrations are 0, 0.16, 0.8, or 4 ng/ml. In triplicate, 50 ul aliquots of these diluted serum-toxin mixtures are added to 150 ul aliquots of SP2-Tac cells (40,000/well in DMEM+10% FBS) in 96-well flat-bottom plates. After incubating the cells for 16-20 hours at 37C, the cells are pulsed for 4-5 hours with $\{^3H\}$-leucine, harvested, and counted to determine inhibition of protein synthesis. Percent neutralization is calculated by determining the % inhibition of protein synthesis of toxin in HSA-PBS, minus the % inhibition in serum, divided by the % inhibition in HSA-PBS, multiplied by 100. For example, if the 1000 ng/ml concentration of LMB-2 + HSA-PBS caused 75% inhibition of SP2/Tac cells and this concentration in serum caused 50% inhibition, the % Neutralization would be 33%.

4.3.3 Analysis of additional research blood

Other blood samples (≤25 ml per 4 weeks) may be drawn for research purposes. There will be no genetic or germ line testing unless the patient is reconsented.

4.4 ON STUDY EVALUATION

4.4.1 Prior to Each Cycle the following will be done:

- Staging will be completed prior to each cycle. This will include an interim history and physical examination with documentation of measurable disease, stage, and performance status. Physical examination should record the diameter, in two planes, of the largest palpable nodes in each of the following sites: cervical, axillary, supraclavicular, inguinal, and femoral. Physical examination should record liver and spleen size as determined by measurement below the respective costal margin.
- FACS analysis of the peripheral blood will be done.
- Laboratory evaluation to be done on or within 3 days prior to day 1 are: CBC/diff, acute care panel, hepatic panel, mineral panel, LDH, PT, PTT, urinalysis, fibrinogen, D-dimer, uric acid, amylase, lipase, CK, IgG, IgA, IgM, CRP, ferritin, haptoglobin, Beta-2 microglobulin and SPEP, 24 hour urine.
- ECG, CXR.

4.4.2 Routine Tests done during Each Cycle

4.4.2.1 Days 3, 5

CBC/diff, acute care panel, hepatic panel, mineral panel, LDH, urinalysis, uric acid, CK.

4.4.2.2 Weeks 2 and 3

The following labs will be drawn once during weeks 2 and 3: CBC/diff, acute care panel, hepatic panel, and albumin.

4.4.2.3 Follow-Up

For follow-up studies in patients off-treatment, which will not affect eligibility for enrollment or retreatment, a non-radioactive neutralization assay may be used, under non-CLIA conditions. For additional correlation between the 2 assays, serum samples are saved to enable the new assay to be run with historical samples previously tested using the radioactive CLIA assay.

4.4.3 Other tests that will be done at specific times:

- Bone marrow biopsy will be done 8 weeks after the patient meets clinical criteria for a CR. A bone marrow biopsy may also be done (but is not required) at the time that clinical CR is documented. Research samples at the time of bone marrow aspirate include sodium heparin (green top) tubes for MRD analysis and flow cytometry, and an EDTA tube for PCR (molecular pathology). Research blood samples at the time of a bone marrow will include flow cytometry, DNA and RNA PaxGene tubes for molecular analysis, and 2 serum separator tubes for neutralizing antibodies and soluble tumor markers.
- CT/MRI scan will be done 8 weeks after the patient meets clinical criteria for a CR. A CT/MRI scan may also be done (but is not required) at the time that clinical CR is documented. If no lymph nodes are found prior to enrollment, an ultrasound rather than CT/MRI may be done at the discretion of the PI.

4.5 COST AND COMPENSATION

4.5.1 Costs

NIH does not bill health insurance companies or participants for any research or related clinical care that participants receive at the NIH Clinical Center. If some tests and procedures performed outside the NIH Clinical Center, participants may have to pay for these costs if they are not covered by insurance company. Medicines that are not part of the study treatment will not be provided or paid for by the NIH Clinical Center.

4.5.2 Compensation

Participants will not be compensated on this study.

4.5.3 Reimbursement

The NCI will cover the costs of some expenses associated with protocol participation. Some of these costs may be paid directly by the NIH and some may be reimbursed to the participant/guardian as appropriate. The amount and form of these payments are determined by the NCI Travel and Lodging Reimbursement Policy.

4.6 TOXICITY CRITERIA

CTCAE term (AE description) and grade: The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 3.0 will be utilized until December 31, 2010 for AE reporting. CTCAE version 4.0 will be utilized beginning January 1, 2011. All appropriate treatment areas should have access to a copy of the CTCAE version 4.0. A copy of the CTCAE version 4.0 can be downloaded from the CTEP web site (http://ctep.cancer.gov).

4.7 RETREATMENT

Patients without evidence of progression and without sufficient neutralizing antibodies will be eligible to receive retreatment cycles of LMB-2 at the same dose with at least 25 days between cycles. Patients will be tested for neutralizing antibodies prior to each cycle. Treatment may not continue for beyond two cycles for a complete response (CR), beyond four cycles for CR with minimal residual disease (MRD), or beyond six cycles for a partial response (PR) or stable disease (SD) without prior FDA approval.

4.8 SUPPORTIVE CARE

- Allergic reaction will be treated acutely with antihistamines (including diphenhydramine, hydroxyzine, & ranitidine), fluids, bronchodilators, and/or epinephrine.
- Nausea and Vomiting: Patients who develop nausea will be treated with a serotonin 5-HT receptor inhibitor for at least 24 hours after their last episode of nausea. Other antiemetics such as prochlorperazine, metoclopramide, or lorazepam may be used in addition if necessary.
- Myalgias: Patients who develop myalgias may be given acetaminophen 650 to 1000 mg every 6 hours until 24 hours after completing the last dose of LMB-2. It may then be given as needed. Patients may receive NSAIDs or opioid analgesics if acetaminophen is inadequate.
- Vascular leak syndrome/Capillary leak syndrome: supportive care may include fluid and electrolyte management, diuresis, albumin, and cardiovascular support.
- Hypotension: Patients will be encouraged to increase oral fluid intake. In addition, for an orthostatic SBP change of >20 mm Hg and an absolute SBP of <100 mm Hg, an IVF bolus may be given as deemed clinically appropriate. Refractory hypotension may require treatment in the intensive care unit with pressors.
- Fever: Patients who develop temperatures >38.0° C may receive scheduled acetaminophen 650 to 1000 mg every 6 hours until 24 hours after completing the last dose of LMB-2. It may then be given as needed.
- Thrombocytopenia should be treated conservatively. In the absence of bleeding or a planned invasive procedure, platelet transfusions should only be given for a platelet count below 10,000. If invasive procedures are planned or the patient develops bleeding, platelet transfusions should be administered in accordance with standard of practice, usually maintaining a platelet count > 50,000/mm^3.
- Symptomatic anemia should be treated with appropriate red blood cell support. Transfusion is recommended if the hemoglobin falls below 8g/dL. Recombinant erythropoietin may be also be used.

- Febrile Neutropenia is a life-threatening complication requiring hospitalization and urgent broad-spectrum antibiotics. Hematopoietic growth factors may be used if clinically indicated. Such cases will be evaluated individually to determine the toxicity grade. Neutropenia due to LMB-2 is not expected.
- Central venous access devices such as a temporary internal jugular or subclavian lines, PICC lines, semi-permanent HICKMAN, Groshong catheters, or medi-port implanted devices can all be used in this study. All devices will have nursing supervision and include patient self-care instruction.
- Nutritional assessment and psychological support: Refractory neoplasms are commonly complicated by malnutrition. Patients with weight loss or evidence of wasting syndrome should have a nutritional consult. When necessary, social Work will be proactively involved with these patients' biopsychosocial well-being.

4.9 CRITERIA FOR REMOVAL FROM PROTOCOL THERAPY AND OFF STUDY CRITERIA

Prior to removal from study, effort must be made to have all subjects complete a safety visit approximately 30 days following the last dose of study therapy.

4.9.1 Criteria for removal from protocol therapy

- Progression of disease during active treatment on study protocol.
- Grade III Allergic Reaction and grade II urticaria despite premedication.
- More than 2 dose reductions are required.
- Patient non-compliance or voluntary withdrawal.
- Patient's serum neutralizes > 75% of the activity of 1 μg/mL of LMB-2.
- Grade IV toxicity other than hematologic and CPK.
- Grade IV hematologic toxicity lasting > 5 days.
- Grade IV CPK associated with any other DLT or not resolving to < grade II within 2 weeks.

4.10 POST TREATMENT EVALUATION (OFF TREATMENT BUT ON STUDY)

Patients will be taken off treatment when they meet off treatment criteria. Patients who are off treatment due to toxicity will be followed until resolution of their side effects. On- or off-treatment patients who have not had progressive disease will be followed every 3-12 months until they have progressive disease (see Section **12**, Study Calendar). Patients who have progressive disease may also need follow-up for other endpoints, including neutralizing antibodies (see Section **12**, Study Calendar) . Follow-up assessments will be conducted per PI discretion and may be obtained from clinic visits either at NIH or elsewhere.

4.11 OFF-STUDY CRITERIA

- Screen failure
- Patient begins another LMB trial which allows data collection
- Unwillingness to continue follow-up or provide follow-up data.

4.12 LOST TO FOLLOW-UP

A participant will be considered lost to follow-up if he or she fails to return for 3 consecutive scheduled visits and is unable to be contacted by the study site staff.
The following actions must be taken if a participant fails to return to the clinic for a required study visit:

- The site will attempt to contact the participant and reschedule the missed visit within 2 weeks and counsel the participant on the importance of maintaining the assigned visit schedule and ascertain if the participant wishes to and/or should continue in the study.
- Before a participant is deemed lost to follow-up, the investigator or designee will make every effort to regain contact with the participant (where possible, 3 telephone calls and, if necessary, a certified letter to the participant's last known mailing address or local equivalent methods). These contact attempts should be documented in the participant's medical record or study file.
- Should the participant continue to be unreachable, he or she will be considered to have withdrawn from the study with a primary reason of lost to follow-up.

5 HUMAN SUBJECTS PROTECTIONS

5.1 RATIONALE FOR SUBJECT SELECTION

Selection is based on gender, ethnic background or race.
Subjects from both genders and all racial/ethnic groups are eligible for this study if they meet the eligibility criteria. To date, there is no information that suggests that differences in drug metabolism or disease response would be expected in any one patient group. Efforts will be made to extend accrual to a representative population, but in this preliminary study, a balance must be struck between patient safety considerations and limitations on the number of patients exposed to a potentially toxic treatment on the one hand and the need to explore gender and ethnic aspects of clinical research on the other. If differences in the outcome which correlate with gender or ethnic identity are noted, accrual may be expanded or a follow-up study may be written to investigate these differences.

5.1.1.1 Justification for Exclusions

Patients with hepatitis C positivity are excluded from this trial because of the concern that LMB-2 may worsen hepatitis C. Patients with hepatitis B are not excluded if on Lamivudine, which is used to prevent reactivation/worsening of hepatitis B with chemotherapy and other immunosuppressive treatments. Patients that are pregnant or breast-feeding will be excluded from this trial because the effect of LMB-2 on the developing fetus or the nursing infant is unknown and potentially harmful.

5.2 PARTICIPATION OF CHILDREN

Only patients 18 years of age or older will be enrolled on this study, since the safety of this agent has not been previously defined in a pediatric population.

5.3 PARTICIPATION OF SUBJECTS UNABLE TO CONSENT

Adults unable to give consent are excluded from enrolling in the protocol. However, re-consent may be necessary and there is a possibility, though unlikely, that subjects could become

decisionally impaired. For this reason and because there is a prospect of direct benefit from research participation (section **5.5**), all subjects will be offered the opportunity to fill in their wishes for research and care, and assign a substitute decision maker on the "NIH Advance Directive for Health Care and Medical Research Participation" form so that another person can make decisions about their medical care in the event that they become incapacitated or cognitively impaired during the course of the study. Note: The PI or AI will contact the NIH Ability to Consent Assessment Team(ACAT) for evaluation to assess ongoing capacity of the subjects and to identify an LAR, as needed.

Please see Section **5.6.1** for consent procedure.

5.4 EVALUATION OF BENEFITS AND RISKS/DISCOMFORTS

5.4.1 Potential benefits to subjects expected from the trial

Patients will receive evaluation and treatment of their tumor at the National Cancer Institute's Clinical Center. This protocol may or may not benefit an individual, but the results may help the investigators learn more about the disease and develop new treatments for patients with this disease. Benefit cannot be promised nor can the chance of benefit be accurately predicted. This research treatment is unlikely to be curative but may offer temporary control of the disease.

5.4.2 Alternative approaches or treatments

Patients will be consented verbally and in writing regarding the risks and benefits of this trial, the treatment requirements, and alternative approaches to entering on this trial.

5.4.3 Procedures for protecting against or minimizing any potential risks

All care will be taken to minimize side effects, but they can be unpredictable in nature and severity. This study may involve risks to patients which are currently unforeseeable. Patients will be examined and evaluated prior to enrollment and prior to each cycle. The Clinical Center staff will observe all patients during the drug administration. All evaluations to monitor the treatment of patients will be recorded in the patient chart. Patients are required to have a local physician to improve long-term care and to monitor for complications. They will have blood draws at home to monitor side effects. If patients suffer any physical injury as a result of the participation in this study, immediate medical treatment is available at the Clinical Center, National Cancer Institute, Bethesda, Maryland. Although no compensation is available, any injury will be evaluated and treated in keeping with the benefits or care to which patients are entitled under applicable regulations.

5.4.4 Identified Risks to Study Procedures

5.4.4.1 Blood Sampling

Side effects of blood draws include pain and bruising, lightheadedness, and rarely, fainting.

5.4.4.2 Imaging

In addition to the radiation risks discussed below, CT scans may include the risks of an allergic reaction to the contrast. Participants might experience hives, itching, headache, difficulty breathing, increased heartrate and swelling.

5.4.4.3 MR Imaging

The risks of MR imaging are relatively small.
The US Food and Drug Administration has issued warnings that administration of gadolinium (updated September 9, 2010), the MRI contrast imaging agent used in this protocol, has been associated with development of a disease called **nephrogenic systemic fibrosis (NSF)**. The syndrome is rare (approximately 600 cases reported worldwide as of September 2010, out of several million administrations of gadolinium), but disabling and in some cases, fatal. All cases to date have occurred in patients with severe renal disease, including patients on dialysis. NSF has been nearly eradicated secondary to careful screening of renal function and avoiding use of gadolinium in patients with eGFR <30 ml/min/1.73 BSA. Even in patients with end stage renal disease, there have been only rare occurrences of NSF because of precautions taken to use more stable contrast agents at lower doses. This protocol excludes patients with severe renal insufficiency (eGFR <30 ml/min/1.73 BSA). The FDA has issued warnings in 2017 and 2018 that some gadolinium may be retained in the brain, bone and skin although health risks of accumulation have not been reported to date. In accordance with the FDA Drug Safety Communication of 05/16/2018, the Medication Guide for gadobutrol (or other macrocyclic gadolinium contrast agent if applicable) will be made available to all subjects with scans that will involve gadolinium-based contrast agent administration.

5.4.5 Risks from Radiation Exposure

On this study, participants will receive up to 4 CT scans/year The total radiation dose for research purposes will be approximately 5.2 rem. The risk of getting cancer from the radiation exposure in this study is 0.5% and of getting a fatal cancer is 0.3%.

5.4.6 Provisions for monitoring data collection to ensure safety of subjects

As information is gathered from this trial, clinical results will be shared with patients while maintaining patient confidentiality. Laboratory and clinical data will be frequently gathered and any new significant findings found during the course of the research, which may affect a patient's willingness to participate further, will be explained. Moreover, in all publications and presentations resulting from this trial, patients' anonymity will be protected to the maximum extent possible. Authorized personnel from the National Cancer Institute (NCI) and Food and Drug Administration (FDA) may have access to research files in order to verify that patients' rights have been safeguarded. In addition, patient names will be given to the Central Registration to register and verify patients' eligibility.

Primary and final analyzed data will have identifiers so that research data can be attributed to an individual human subject participant.

End of study procedures: Data will be stored according to HHS, FDA regulations and NIH Intramural Records Retention Schedule as applicable.

5.5 RISKS/BENEFITS ANALYSIS

Patients enrolled on this study will be those with a disease that is considered incurable. They will generally have a poor prognosis and have no standard treatment options known to significantly improve survival. Thus, patients may experience significant treatment related morbidity, and/or have progressive complications of their disease. Although LMB-2 is an experimental new immunotoxin, another drug with a similar mechanism of action and similar toxicities was recently approved for CTCL. In addition, we have seen responses to LMB-2 in patients with CLL and HCL with acceptable toxicities. Consequently, we believe that patients participating in this trial will have an acceptable benefit/risk ratio.

5.6 CONSENT PROCESS AND DOCUMENTATION

The informed consent document will be provided as a physical or electronic document to the participant or consent designee(s) as applicable for review prior to consenting. A designated study investigator will carefully explain the procedures and tests involved in this study, and the associated risks, discomforts and benefits. In order to minimize potential coercion, as much time as is needed to review the document will be given, including an opportunity to discuss it with friends, family members and/or other advisors, and to ask questions of any designated study investigator. A signed informed consent document will be obtained prior to entry onto the study.

The initial consent process as well as re-consent, when required, may take place in person or remotely (e.g., via telephone or other NIH approved remote platforms used in compliance with policy, including HRPP Policy 303) per discretion of the designated study investigator and with the agreement of the participant/consent designee(s). Whether in person or remote, the privacy of the subject will be maintained. Consenting investigators (and participant/consent designee, when in person) will be located in a private area (e.g., clinic consult room). When consent is conducted remotely, the participant/consent designee will be informed of the private nature of the discussion and will be encouraged to relocate to a more private setting if needed.

Consent will be documented with required signatures on the physical document (which includes the printout of an electronic document sent to participant) or as described below, with a manual (non-electronic) signature on the electronic document. When required, witness signature will be obtained similarly as described for the investigator and participant.

Manual (non-electronic) signature on electronic document:

When a manual signature on an electronic document is used for the documentation of consent at the NIH Clinical Center, this study will use the following to obtain the required signatures:

- Adobe platform (which is not 21 CFR Part 11 compliant); or,
- iMedConsent platform (which is 21 CFR Part 11 compliant)

During the consent process, participants and investigators will view individual copies of the approved consent document on screens at their respective locations (if remote consent); the same screen may be used when in the same location but is not required.

Both the investigator and the participant will sign the document using a finger, stylus or mouse.

Note: Refer to the CCR SOP PM-2, Obtaining and Documenting the Informed Consent Process for additional information (e.g., verification of participant identity when obtaining consent remotely) found at: https://ccrod.cancer.gov/confluence/pages/viewpage.action?pageId=73203825.

5.6.1 Consent Process for Adults Who Lack Capacity to Consent to Research Participation

For participants addressed in Section **5.3**, an LAR will be identified consistent with Policy 403 and informed consent obtained from the LAR, as described in Section **5.6**.

5.6.2 Consent to obtain samples for eligibility

The patient must give informed consent even before protocol enrollment, at the time that samples are taken to test eligibility. A separate informed consent is given to the patient clarifying what tests will be done and stating that no germline testing is to be done. This eligibility informed consent also states that the patient may eventually not be considered a candidate for the study. The eligibility consent may be signed by the patient prior to arrival at NIH, but the actual treatment (standard) consent must be signed in person prior to protocol enrollment.

5.7 SAMPLE STORAGE, TRACKING AND DISPOSITION

5.7.1.1 Description of data/specimens: Blood, bone marrow, lymph node, skin, and other tumor samples.

5.7.1.2 Research being conducted: Samples to be saved for additional tests:

- Neutralizing antibodies: Antibodies a patient might make which block the effect of certain recombinant immunotoxins like LMB-2. Requires about 6 ml of blood.
- Flow cytometry assays to quantify tumor markers on the malignant cells. Requires about 3 ml of blood.
- Bone marrow biopsy samples, whether they obtained at NIH or elsewhere, and whether the bone marrow test has already been done or not yet done.
- Cytotoxicity assays. Leukemia cells from the blood, bone marrow, or other tissues may be tested with LMB-2 and related drugs to determine if the malignant cells can be killed outside the body. Requires 15-45 ml of blood.
- Soluble CD25, CD22, and other tumor markers: To estimate the amount of cancer cells in the body by measuring proteins which fall off cancer cells and go into the blood. Requires about 6 ml of blood.
- HLA typing to better understand the immune system in patients with hairy cell leukemia. Requires about 6 ml of blood.
- PAX-gene tube: To obtain RNA or DNA to study the mechanism of how leukemia cells form, and to detect very low levels of leukemia cells in patients. Requires about 3 ml of blood.
- RNA samples can also be used, in an assay called micro-arrays, to study why some patients may not respond as well as others to recombinant immunotoxins like LMB-2. Taken with PaxGene tube.

- Samples of blood to study how hemolytic uremic syndrome (HUS), a major toxicity of a recombinant immunotoxin called BL22, which is similar to LMB-2, occurs and might be prevented. Requires about 3 ml of blood.
- DNA samples to look for abnormalities which might make a patient more susceptible to HUS. Requires about 3 ml of blood.
- Assays which could have an impact on both patients and their children, including studies of genetic cancer risk, will not be done.
- Samples to determine levels of immunotoxin in blood, urine, and other tissues.

5.7.1.3 Timeframe and location of storage: Samples will be stored and cataloged longer than a year, in alarmed freezers at our Leidos Biomedical Research, Inc. contract lab in Frederick, MD, where neutralizing antibodies and PK samples are tested. Portions of samples which are stored at Leiodos Biomedical Research, Inc. in Frederick may also be stored and tested in the LMB lab (Building 37) for longer than a year providing there is sample remaining after studies are done. All samples will be stored with unique patient numbers and without personal identifiers. After closure of the protocol, the samples will either be destroyed or their storage and use will be governed by a subsequent protocol. Samples at Leiodos Biomedical Research, Inc. in Frederick will be tracked in a secure electronic database and the PI will report lost or destroyed samples per the requirements of Section **8.2**.

6 DOSING DELAYS/DOSE MODIFICATION

6.1 NO DLT

Continue treatment or retreatment at same dosage.

6.2 DLT

This also applies to patients undergoing retreatment cycles.

6.2.1 Off Treatment for DLT from:

- Grade III Allergic Reaction and grade II urticaria despite premedication.
- Grade IV toxicity other than hematologic and CPK.
- Grade IV hematologic toxicity lasting > 5 days.
- Grade IV CPK associated with any other DLT or not resolving to < grade II within 2 weeks.

6.2.2 Requiring dose reduction if retreating after DLT from:

- Grade III toxicities except for AST, ALT, GGT, and hematologic.
- Grade IV CPK not associated with any other DLT and resolving to < grade II within 2 weeks.

6.3 DOSE DELAY

Dosing may be delayed to evaluate patients without DLT, including those with infections, or with complications of disease or central lines. If LMB-2 therapy is suspended longer than 72 hours during a cycle for toxicity, therapy will not be resumed during that cycle.

7 ADVERSE EVENTS: LIST AND REPORTING REQUIREMENTS (SPONSOR)

Adverse event (AE) monitoring and reporting is a routine part of every clinical trial. The following list of AEs (Section 7.1) and the characteristics of an observed AE (Section 7.2) will determine whether the event requires expedited reporting (via CTEP-AERS) **in addition** to routine reporting.

7.1 COMPREHENSIVE ADVERSE EVENTS AND POTENTIAL RISKS LIST(S) (CAEPRS)

The Comprehensive Adverse Event and Potential Risks list (CAEPR) provides a single list of reported and/or potential adverse events (AE) associated with an agent using a uniform presentation of events by body system. In addition to the comprehensive list, a subset, the Specific Protocol Exceptions to Expedited Reporting (SPEER), appears in a separate column and is identified with bold and italicized text. This subset of AEs (SPEER) is a list of events that are protocol specific exceptions to expedited reporting to NCI via CTEP-AERS (except as noted below). Refer to the 'CTEP, NCI Guidelines: Adverse Event Reporting Requirements' http://ctep.cancer.gov/protocolDevelopment/adverse_effects.htm for further clarification. The CAEPR does not provide frequency data; refer to the Investigator's Brochure for this information. Below is the CAEPR for LMB-2 (Anti-Tac[Fv]-PE-38).
NOTE: Report AEs on the SPEER **ONLY IF** they exceed the grade noted in parentheses next to the AE in the SPEER. If this CAEPR is part of a combination protocol using multiple investigational agents and has an AE listed on different SPEERs, use the lower of the grades to determine if expedited reporting is required.

7.1.1 Comprehensive Adverse Events and Potential Risks List (CAEPR) for LMB-2 Immunotoxin (Anti-Tac[Fv]-PE-38, NSC 676422)

Version 1.3, July 26, 2013[1]

Adverse Events with Possible Relationship to LMB-2 (Anti-Tac[Fv]-PE-38) (CTCAE 4.0 Term)	Specific Protocol Exceptions to Expedited Reporting (SPEER)
BLOOD AND LYMPHATIC SYSTEM DISORDERS	
Anemia	
CARDIAC DISORDERS	
Left ventricular systolic dysfunction	***Left ventricular systolic dysfunction (Gr 2)***
Pericardial effusion	***Pericardial effusion (Gr 2)***
Sinus tachycardia	
GASTROINTESTINAL DISORDERS	
Abdominal distension	
Diarrhea	
Nausea	***Nausea (Gr 2)***
Vomiting	***Vomiting (Gr 2)***
GENERAL DISORDERS AND ADMINISTRATION	
Edema face	***Edema face (Gr 2)***
Edema limbs	***Edema limbs (Gr 2)***
Fatigue	***Fatigue (Gr 2)***
Fever	***Fever (Gr 2)***
IMMUNE SYSTEM DISORDERS	
Allergic reaction	***Allergic reaction (Gr 2)***

Adverse Events with Possible Relationship to LMB-2 (Anti-Tac[Fv]-PE-38) (CTCAE 4.0 Term)	Specific Protocol Exceptions to Expedited Reporting (SPEER)
INVESTIGATIONS	
Alanine aminotransferase increased	***Alanine aminotransferase increased (Gr 2)***
Alkaline phosphatase increased	
Aspartate aminotransferase increased	***Aspartate aminotransferase increased (Gr 2)***
CPK increased	
Creatinine increased	***Creatinine increased (Gr 2)***
GGT increased	
Platelet count decreased	***Platelet count decreased (Gr 2)***
Weight gain	***Weight gain (Gr 2)***
METABOLISM AND NUTRITION DISORDERS	
Anorexia	
Hypoalbuminemia	***Hypoalbuminemia (Gr 2)***
MUSCULOSKELETAL AND CONNECTIVE TISSUE	
Back pain	
Chest wall pain	
Myalgia	***Myalgia (Gr 2)***
NERVOUS SYSTEM DISORDERS	
Dizziness	
Headache	***Headache (Gr 2)***
RENAL AND URINARY DISORDERS	
Hematuria	***Hematuria (Gr 2)***
Proteinuria	***Proteinuria (Gr 2)***
RESPIRATORY, THORACIC AND MEDIASTINAL	
Dyspnea	
Pleural effusion	
VASCULAR DISORDERS	
Capillary leak syndrome	***Capillary leak syndrome (Gr 2)***
Hypotension	***Hypotension (Gr 2)***

[1]This table will be updated as the toxicity profile of the agent is revised. Updates will be distributed to all Principal Investigators at the time of revision. The current version can be obtained by contacting PIO@CTEP.NCI.NIH.GOV. Your name, the name of the investigator, the protocol and the agent should be included in the e-mail.

[2] Infection includes all 75 sites of infection under the INFECTIONS AND INFESTATIONS SOC.

[3]Muscle weakness includes Generalized muscle weakness, Muscle weakness left-sided, Muscle weakness lower limb, Muscle weakness right-sided, Muscle weakness trunk, and Muscle weakness upper limb under the MUSCULOSKELETAL AND CONNECTIVE TISSUE DISORDERS SOC.

Also reported on LMB-2 (Anti-Tac[Fv]-PE-38) trials but with the relationship to LMB-2 (Anti-Tac[Fv]-PE-38) still undetermined:

CARDIAC DISORDERS - Acute coronary syndrome; Myocardial infarction; Paroxysmal atrial tachycardia; Restrictive cardiomyopathy; Supraventricular tachycardia
EYE DISORDERS - Blurred vision; Eye pain
GASTROINTESTINAL DISORDERS - Dyspepsia
GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS - Chills; Non-cardiac chest pain
IMMUNE SYSTEM DISORDERS - Anaphylaxis
INFECTIONS AND INFESTATIONS – Infection[2]
INJURY, POISONING AND PROCEDURAL COMPLICATIONS - Fall
INVESTIGATIONS - Blood bilirubin increased; Cardiac troponin I increased; Fibrinogen decreased; Weight loss
METABOLISM AND NUTRITION DISORDERS - Hypokalemia
MUSCULOSKELETAL AND CONNECTIVE TISSUE DISORDERS - Bone pain; Muscle weakness[3]; Musculoskeletal and connective tissue disorder - Other (acute rhabdomyolysis); Neck pain; Pain in extremity
NERVOUS SYSTEM DISORDERS - Dysgeusia; Presyncope; Syncope; Vasovagal reaction
RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS - Allergic rhinitis; Pneumonitis
SKIN AND SUBCUTANEOUS TISSUE DISORDERS - Hyperhidrosis; Palmar-plantar erythrodysesthesia syndrome; Pruritus; Rash maculo-papular
VASCULAR DISORDERS - Hypertension

Animal Data: The following toxicities have been observed in animal studies with LMB-2 (Anti-Tac[Fv]-PE-38):
leukocytosis

Note: LMB-2 (Anti-Tac[Fv]-PE-38) in combination with other agents could cause an exacerbation of any adverse event currently known to be caused by the other agent, or the combination may result in events never previously associated with either agent.

7.2 ADVERSE EVENT CHARACTERISTICS

- **CTCAE term (AE description) and grade:** The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 will be utilized for AE reporting. All appropriate treatment areas should have access to a copy of the CTCAE version 4.0. A copy of the CTCAE version 4.0 can be downloaded from the CTEP web site http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm.

- For expedited reporting purposes only:
  - AEs for the agent that are ***bold and italicized*** in the CAEPR (*i.e.*, those listed in the SPEER column, Section **7.1.1**) should be reported through CTEP-AERS only if the grade is above the grade provided in the

SPEER.

- Other AEs for the protocol that do not require expedited reporting are outlined in section **Error! Reference source not found.**

- **Attribution** of the AE:
  - Definite – The AE *is clearly related* to the study treatment.
  - Probable – The AE *is likely related* to the study treatment.
  - Possible – The AE *may be related* to the study treatment.
  - Unlikely – The AE *is doubtfully related* to the study treatment.
  - Unrelated – The AE *is clearly NOT related* to the study treatment.

7.3 EXPEDITED ADVERSE EVENT REPORTING

7.3.1 Expedited AE reporting for this study must use CTEP-AERS (CTEP Adverse Event Reporting System), accessed via the CTEP Web site (http://ctep.cancer.gov). The reporting procedures to be followed are presented in the "NCI Guidelines for Investigators: Adverse Event Reporting Requirements for DCTD (CTEP and CIP) and DCP INDs and IDEs" which can be downloaded from the CTEP Web site (http://ctep.cancer.gov). These requirements are briefly outlined in the tables below (Section **7.3.2**).

In the rare occurrence when Internet connectivity is lost, a 24-hour notification is to be made to CTEP by telephone at 301-897-7497. Once Internet connectivity is restored, the 24-hour notification phoned in must be entered electronically into CTEP-AERS by the original submitter at the site.

7.3.2 Expedited Reporting Guidelines

Note: A death on study requires both routine and expedited reporting regardless of causality, unless as noted below. Attribution to treatment or other cause must be provided.

Use the NCI protocol number and the protocol-specific patient ID assigned during trial registration on all reports.

Phase 2 and 3 Trials Utilizing an Agent under a CTEP IND: Reporting Requirements for Adverse Events That Occur Within 30 Days[1] of the Last Dose of the Investigational Agent

	Grade 1	Grade 2	Grade 2	Grade 3	Grade 3	Grades 4 & 5[2]	Grades 4 & 5[2]
	Unexpected and Expected	Unex-pected	Expected	Unexpected	Expected	Unex-pected	Expected

				with Hospitali-zation	without Hospitali- zation	with Hospitali-zation	without Hospitali-zation		
Unrelated Unlikely	Not Required	Not Required	Not Required	10 Calendar Days	Not Required	10 Calendar Days	Not Required	10 Calendar Days	10 Calendar Days
Possible Probable Definite	Not Required	10 Calendar Days	Not Required	10 Calendar Days	10 Calendar Days	10 Calendar Days	Not Required	24-Hour; 5 Calendar Days	10 Calendar Days

1 Adverse events with attribution of possible, probable, or definite that occur greater than 30 days after the last dose of treatment with an agent under a CTEP IND require reporting as follows:
CTEP-AERS 24-hour notification followed by complete report within 5 calendar days for:
· Grade 4 and Grade 5 unexpected events
CTEP-AERS 10 calendar day report:
· Grade 3 unexpected events with hospitalization or prolongation of hospitalization
· Grade 5 expected events
2 Although an CTEP-AERS 24-hour notification is not required for death clearly related to progressive disease, a full report is required as outlined in the table.
December 15, 2004

7.4 DATA COLLECTION AND EVALUATION

All observed or volunteered adverse events, regardless of treatment group or suspected causal relationship to study drug, will be recorded into a 21 CFR Part 11-compliant data capture system . Adverse events must be reported in a consistent manner for all patients on study, based on the following rules: Adverse events will be identified and graded using the NCI CTCAE version 3 until December 31, 2010. CTCAE version 4.0 will be utilized beginning January 1, 2011. Next, it will be assessed if the adverse event is related to the medical treatment (attribution). If so, it will be determined whether the adverse event is expected or unexpected (see Investigator's Brochure and section **7.4.1**). If, in the judgment of the PI, the adverse event is not constant but fluctuates (or stutters) or changes grade during a period of time, it may be reported as one event with the grade being the maximum grade reached, and the resolved date being the date it returns to baseline grade. Clinical judgment of the PI must be used to assess the baseline grade of adverse events. The laboratory value immediately before beginning drug is usually used to determine grades of baseline laboratory AEs, but laboratory values from the prior 90 days may be used if clinically relevant. All calcium values will be corrected for albumin. To determine the corrected calcium in mmol/L, subtract the albumin in g/dL from 4.0, multiply the result by 0.2, then add the product to the measured calcium in mmol/L.

7.4.1 Expected Adverse Events

7.4.1.1 Grade 4: none

7.4.1.2 Grade 3: AST, SGOT, ALT, SGPT, GGT, Albumin, serum-low (hypoalbuminemia), platelets.

7.4.1.3 Grade 2:

- Blood Bone Marrow: platelets, hemoglobin, Neutrophils/granulocytes (ANC/AGC), Leukocytes (total WBC), lymphopenia.
- Cardiovascular: Acute Vascular Leak syndrome/Capillary Leak syndrome (CTEP defined section **4.2.2**), edema, hypotension, pericardial effusion/pericarditis, PTT, INR (International Normalized Ratio of prothrombin time).
- Constitutional: fatigue, fever, weight gain.
- GI: nausea, vomiting, diarrhea.
- Hepatic: AST, SGOT, ALT, SGPT, GGT, Albumin, serum-low (hypoalbuminemia), alkaline phosphatase.
- Metabolic/Laboratory: bicarbonate, CPK, Calcium, serum-low (hypocalcemia), Potassium, serum-low (hypokalemia), Magnesium, serum-low (hypomagnesemia), Sodium, serum-low (hyponatremia), Phosphate, serum-low (hypophosphatemia).
- Musculoskeletal: muscle weakness.
- Pain: myalgia.
- Renal: creatinine, proteinuria.

All Adverse Events must be reported in routine study data submissions. AEs reported through CTEP-AERS must also be reported in routine study data submissions.

8 NIH REPORTING REQUIREMENTS / DATA AND SAFETY MONITORING PLAN

8.1 DEFINITIONS

Please refer to definitions provided in Policy 801: Reporting Research Events found at: https://irbo.nih.gov/confluence/pages/viewpage.action?pageId=36241835#Policies&Guidance-800Series-ComplianceandResearchEventReportingRequirements..

8.2 OHSRP OFFICE OF COMPLIANCE AND TRAINING / IRB REPORTING

8.2.1 Expedited Reporting

Please refer to the reporting requirements in Policy 801: Reporting Research Events and Policy 802 Non-Compliance Human Subjects Research found at: https://irbo.nih.gov/confluence/pages/viewpage.action?pageId=36241835#Policies&Guidance-800Series-ComplianceandResearchEventReportingRequirements.. Note: Only IND Safety Reports that meet the definition of an unanticipated problem will need to be reported per these policies.

8.2.2 IRB Requirements for PI Reporting at Continuing Review

Please refer to the reporting requirements in Policy 801: Reporting Research Events found at: https://irbo.nih.gov/confluence/pages/viewpage.action?pageId=36241835#Policies&Guidance-800Series-ComplianceandResearchEventReportingRequirements..

8.3 NCI Clinical Director Reporting

Problems expeditiously reviewed by the OHSRP in the NIH eIRB system will also be reported to the NCI Clinical Director/designee; therefore, a separate submission for these reports is not necessary.

In addition to those reports, all deaths that occur within 30 days after receiving a research intervention should be reported via email unless they are due to progressive disease.

To report these deaths, please send an email describing the circumstances of the death to NCICCRQA@mail.nih.gov within one business day of learning of the death.

8.4 NIH Required Data and Safety Monitoring Plan

The clinical research team will meet on a regular basis (at least weekly) when participants are being actively treated on the trial to discuss each participant. Decisions about dose level enrollment and dose escalation if applicable will be made based on the toxicity data from prior participants.

All data will be collected in a timely manner and reviewed by the principal investigator or a lead associate investigator. Events meeting requirements for expedited reporting as described in Section **8.2.1** will be submitted within the appropriate timelines.

The principal investigator will review adverse event and response data on each participant to ensure safety and data accuracy. The principal investigator will personally conduct or supervise the investigation and provide appropriate delegation of responsibilities to other members of the research staff.

9 REGULATORY AND OPERATIONAL CONSIDERATIONS

9.1 Study Discontinuation and Closure

This study may be temporarily suspended or prematurely terminated if there is sufficient reasonable cause. Written notification, documenting the reason for study suspension or termination, will be provided by the suspending or terminating party to study participants, investigator, the Investigational New Drug (IND) sponsor and regulatory authorities. If the study is prematurely terminated or suspended, the Principal Investigator (PI) will promptly inform study participants, and the Institutional Review Board (IRB), and will provide the reason(s) for the termination or suspension. Study participants will be contacted, as applicable, and be informed of changes to study visit schedule.

Circumstances that may warrant termination or suspension include, but are not limited to:

- Determination of unexpected, significant, or unacceptable risk to participants
- Demonstration of efficacy that would warrant stopping

- Insufficient compliance to protocol requirements
- Data that are not sufficiently complete and/or evaluable
- Determination that the primary endpoint has been met
- Determination of futility

Study may resume once concerns about safety, protocol compliance, and data quality are addressed, and satisfy the IRB and as applicable, Food and Drug Administration (FDA).

9.2 Quality Assurance and Quality Control

The clinical site will perform internal quality management of study conduct, data and biological specimen collection, documentation and completion. An individualized quality management plan will be developed to describe a site's quality management.
Quality control (QC) procedures will be implemented beginning with the data entry system and data QC checks that will be run on the database will be generated. Any missing data or data anomalies will be communicated to the site for clarification/resolution.
Following written Standard Operating Procedures (SOPs), the monitors will verify that the clinical trial is conducted and data are generated and biological specimens are collected, documented (recorded), and reported in compliance with the protocol, International Conference on Harmonisation Good Clinical Practice (ICH GCP), and applicable regulatory requirements (e.g., Good Laboratory Practices (GLP), Good Manufacturing Practices (GMP)).
The investigational site will provide direct access to all trial related sites, source data/documents, and reports for the purpose of monitoring and auditing by the sponsor, and inspection by local and regulatory authorities.

9.3 Conflict of Interest Policy

The independence of this study from any actual or perceived influence, such as by the pharmaceutical industry, is critical. Therefore, any actual conflict of interest of persons who have a role in the design, conduct, analysis, publication, or any aspect of this trial will be disclosed and managed. Furthermore, persons who have a perceived conflict of interest will be required to have such conflicts managed in a way that is appropriate to their participation in the design and conduct of this trial. The study leadership in conjunction with the National Cancer Institute has established policies and procedures for all study group members to disclose all conflicts of interest and will establish a mechanism for the management of all reported dualities of interest.

9.4 Confidentiality and Privacy

Participant confidentiality and privacy is strictly held in trust by the participating investigators, their staff, and the sponsor(s). This confidentiality is extended to cover testing of biological samples and genetic tests in addition to the clinical information relating to participants. Therefore, the study protocol, documentation, data, and all other information generated will be held in strict confidence. No information concerning the study or the data will be released to any unauthorized third party without prior written approval of the sponsor.

All research activities will be conducted in as private a setting as possible.

The study monitor, other authorized representatives of the sponsor, representatives of the Institutional Review Board (IRB), and/or regulatory agencies may inspect all documents and records required to be maintained by the investigator, including but not limited to, medical

records (office, clinic, or hospital) and pharmacy records for the participants in this study. The clinical study site will permit access to such records.

The study participant's contact information will be securely stored at the/each clinical site for internal use during the study. At the end of the study, all records will continue to be kept in a secure location for as long a period as dictated by the reviewing IRB, Institutional policies, or sponsor requirements.

Study participant research data, which is for purposes of statistical analysis and scientific reporting, will be stored at the NCI CCR. This will not include the participant's contact or identifying information. Rather, individual participants and their research data will be identified by a unique study identification number. The study data entry and study management systems used by the clinical site and by NCI CCR research staff will be secured and password protected. At the end of the study, all study databases will be archived at the NIH.

To further protect the privacy of study participants, a Certificate of Confidentiality has been issued by the National Institutes of Health (NIH). This certificate protects identifiable research information from forced disclosure. It allows the investigator and others who have access to research records to refuse to disclose identifying information on research participation in any civil, criminal, administrative, legislative, or other proceeding, whether at the federal, state, or local level. By protecting researchers and institutions from being compelled to disclose information that would identify research participants, Certificates of Confidentiality help achieve the research objectives and promote participation in studies by helping assure confidentiality and privacy to participants.

10 PHARMACEUTICAL INFORMATION

10.1 LMB-2 IS AN INVESTIGATIONAL RECOMBINANT IMMUNOTOXIN.

10.1.1 LMB-2 (NSC 676422)

Other Names: Anti-TAC (Fv) PE 38.

Classification: Recombinant immunotoxin.

Description: LMB-2, a 63-kD single-chain recombinant immunotoxin, is comprised of variable regions of the light and heavy chains (Fv) of a murine monoclonal antibody (anti-TAC) against the 55-kD subunit of the low affinity interleukin-2 receptor (IL-2R) fused to a truncated derivative of *Pseudomonas exotoxin*, (PE 38).

Mode of Action: The human IL-2R (also known as TAC antigen and CD25) plays an important role in lymphocyte differentiation and immune response regulation. It is overexpressed on various types of malignant cells and lymphocytes mediating autoimmune disease, making IL-2R a potential cancer therapy target. Anti-TAC is a murine monoclonal antibody that binds to the IL-2R (with high affinity blocking the interaction of IL-2 with IL-2R. *Pseudomonas exotoxin* (PE) 38 is a truncated portion of a protein secreted by *P. aeruginosa* that lacks the native cell-binding site and kills mammalian cells by catalyzing irreversible ADP-ribosylation and inactivating elongation factor 2, halting protein synthesis. LMB-2 selectively binds to cells bearing IL-2R and is internalized to release PE 38, causing cell destruction.

How Supplied: LMB-2 is available as a sterile frozen solution in phosphate buffered saline and

may be vialed and labeled at different concentrations (i.e. 420 µg/mL, 436 µg/mL, 484 µg/mL, 534 µg/mL, etc.). The pH is approximately 7.4.

Manufacturer: Produced by the Monoclonal Antibody and Recombinant Protein facility, NCI, Frederick, MD.

Please note: The concentration of LMB-2 may vary from lot to lot. Please check the label for the correct concentration prior to the preparation of each dose.

Preparation: Thawing instructions: Warm vials in the hand for 10 to 20 seconds before placing them in a water bath to thaw. Place vials in a cup of room temperature (15-30ºC) sterile water for injection, USP such that when the vial is upright, the water level will be at the neck of the vial. Visually inspect the vials after thawing. Do not use if solution appears turbid. Do not shake; proteins can foam and may denature.

LMB-2 should only be diluted in 0.2% human serum albumin (HSA) in 0.9% sodium chloride. **Please note:** Particulate matter was found in vials from lots 103037 and 103038 during the 60-month stability testing. The lots met all other release specifications, including composition and potency. Tests were conducted with a Millex GV 25 mm (0.2 micron) filter to remove the particulates. Post filtration studies demonstrated minimal loss of potency. **LMB-2 undiluted solution must be filtered with a 0.2 micron low protein binding Millex GV filter prior to adding to the 0.2% HSA in 0.9% sodium chloride.**

IV infusion: The required amount of LMB-2 (436 µg/mL, the undiluted vial) will be diluted with 0.2% HSA in 0.9% sodium chloride to a total volume of 50 mL in an empty Partial Additive Container (PAB®). Filter LMB-2 with a 0.2 micron low protein binding Millex GV filter prior to adding to the 0.2% HSA in 0.9% sodium chloride. Agitate gently to disperse.

A PAB® container is a standard, commonly-used parenteral product container that is composed of an ethylene and propylene co-polymer without plasticizer. It is an empty sterile bag to which pharmacy personnel add the various components specified by the protocol to a specific prescribed volume. It is preferred over other plastic containers because it is manufactured without polyvinylchloride (PVC) and plasticizers such as di-(2-ethylhexyl) phthalate (DEHP) with which some chemotherapy agents interact.

Storage: Intact vials should be stored in the freezer at -70ºC or below. The intravenous admixture should be stored in the refrigerator (2-8ºC). Thawed vials should not be refrozen.

Stability: Intact vials of LMB-2 are stable for at least 5 years when stored at -70ºC. Once thawed, intact vials are stable for 24 hours when stored in the refrigerator (2-8ºC) and for 4 hours when stored at room temperature (15-30ºC). LMB-2 is stable for 25 hours at 2-8ºC once further diluted in 0.2% HSA in 0.9% sodium chloride. Vials cannot be refrozen.

Route(s) of Administration: Intravenous (IV).

Method of Administration: Treatment doses should be infused intravenously over 30 minutes.

Patient Care Implications: If necessary, supportive care for vascular leak syndrome/capillary leak syndrome should be instituted and may include fluid and electrolyte management, diuresis, albumin, glucocorticoids, and cardiovascular support. Other toxicities should be managed clinically.

Anti-emetics or hematologic growth factors are not expected to be required, but are permitted if

indicated. Patients who develop nausea may be treated with a serotonin 5-HT3 receptor antagonist for at least 24 hours after their last episode of nausea. Other antiemetics, such as prochlorperazine, metoclopramide, or lorazepam may be used in addition if necessary.

Patients who develop myalgias may be given acetaminophen 650 mg to 1000 mg every 6 hours until 24 hours after completing the last dose of LMB-2.

It may then be given as needed. Patients may receive NSAIDs or narcotics if acetaminophen is inadequate. Patients who develop temperatures >38.0ºC may receive scheduled acetaminophen 650 mg to 1000 mg every 6 hours until 24 hours after completing the last dose of LMB-2. It may then be given as needed.

Emergency medications should be available in the treatment unit in the event of an anaphylactic reaction. Allergic reactions should be treated acutely with antihistamines, and if needed, with glucocorticoids, fluids, and/or epinephrine.

Agent Ordering and Agent Accountability

NCI supplied agents may be requested by the Principal Investigator (or their authorized designee) at each participating institution. Pharmaceutical Management Branch (PMB) policy requires that agent be shipped directly to the institution where the patient is to be treated. PMB does not permit the transfer of agents between institutions (unless prior approval from PMB is obtained.) The CTEP assigned protocol number must be used for ordering all CTEP supplied investigational agents. The responsible investigator at each participating institution must be registered with CTEP, DCTD through an annual submission of FDA form 1572 (Statement of Investigator), Curriculum Vitae, Supplemental Investigator Data Form (IDF), and Financial Disclosure Form (FDF). If there are several participating investigators at one institution, CTEP supplied investigational agents for the study should be ordered under the name of one lead investigator at that institution.

LMB-2 may be requested by completing a Clinical Drug Request (NIH-986) and mailing it to the Pharmaceutical Management Branch (PMB), DCTD, NCI, 9000 Rockville Pike, EPN Room 7149, Bethesda, MD 20892-7422 or faxing it to (301) 480-4612. For questions call (301) 496-5725.

Agent Inventory Records - The investigator, or a responsible party designated by the investigator, must maintain a careful record of the inventory and disposition of all agents received from DCTD using the NCI Drug Accountability Record (DAR) Form. (See the NCI Investigator's Handbook for Procedures for Drug Accountability and Storage.)

10.1.2 Toxicity

10.1.2.1 Preclinical studies

In a GLP toxicology study, 4 Cynomolgus monkeys received 20 µg/Kg days 1, 3 and 5 with no significant toxicity. Another four monkeys were then given 300 µg/Kg days 1, 3 and 5 and experienced dose-limiting toxicity with anorexia and 2 to 4-fold transaminase elevations. The

LD_{10} and LD_{50} in mice were 200 and 257 μg/Kg every other day for 3 doses. The cause of death was liver damage.

10.1.2.2 Phase I and II trials

Adverse events were reported in relationship to treatment cycle. Grade III-IV toxicities included reversible transaminase elevation, fever, CK elevation, cardiomyopathy, thrombocytopenia, allergic reaction, and diarrhea.
The most common grade I-II toxicities were transaminase elevation, fever, hypoalbuminemia, and fatigue. Other grade I-II toxicities included vascular leak syndrome/capillary leak syndrome, weight gain, hypotension, nausea, pericardial effusion, allergy, proteinuria, and increased creatinine.

10.2 PREMEDICATIONS (ABBREVIATED PHARMACEUTICAL SECTION)

These agents will be provided by the Clinical Center Pharmacy and will be given orally. Please refer to the package inserts for complete pharmaceutical information on these products.

10.2.1 Acetaminophen (Tylenol):

- Side effects are extremely unlikely. Regular use of acetaminophen can cause liver damage especially at high doses (>4000 mg/day or >12 regular strength tablets per day). To minimize this possibility patients should not take over-the-counter products containing acetaminophen during the time periods they are taking scheduled acetaminophen doses on this study.

10.2.2 Ranitidine (Zantac):

- Side effects include tiredness, dizziness, headache, and diarrhea.

10.2.3 Hydroxyzine (Atarax):

- Side effects include sleepiness, dizziness, restlessness, and irritability.

10.2.4 Dexamethasone (Decadron):

- To be given only prior to the 1st dose of retreatment cycles. Side effects of a single dose of dexamethasone might include difficulty sleeping, increased hunger, and increased blood glucose. Steroids are associated with an increased risk of infection although this is more applicable to chronic use.

11 CORRELATIVE/SPECIAL STUDIES

Note: tubes and media for research tests may be substituted based on availability with the permission of the PI or laboratory investigator.

11.1 ASSAY FOR DETECTING NEUTRALIZING ANTIBODIES TO LMB-2.

11.1.1 Set up SP2/Tac cell plates using SP2/Tac cell line protocols. Place cell plates in 37°C incubator until needed.

11.1.2 Using a costar #3799 U-bottom plate, add 72 ul of .2% PBS/HSA to wells A1-A4 and 60 ul to wells A9-A-12. Add patient serum, obtained from serum separator or other clot tube, to the plate in the following manner:

a. Patient 1: 45 ul of serum in wells D1-D4
b. Patient 2: 45 ul of serum in wells E1-E4
c. Patient 3: 45 ul of serum in wells F1-F4
d. Patient 4: 45 ul of serum in wells G1-G4
e. Patient 5: 45 ul of serum in wells H1-H4

11.1.3 Thaw LMB-2 toxin in hand and add 15 ul to well A12. Mix well. Transfer 15 ul from well A12 to well A11. Mix well. Transfer 15 ul from well A11 to well A10. Mix well. Well A9 does not receive toxin.

11.1.4 Using a multichannel pipette, transfer 8 ul from wells A9-A12 into wells A1-A4. Well A1 receives from well A9, well A2 from well A10, etc.

11.1.5 In a similar manner, transfer 5 ul from wells A9-A12 into the patient serum wells. For example, well D1 receives from well A9, etc. Dispel pipette tips between additions to patients samples.

11.1.6 Using a multichannel pipette, mix wells A1-A4, and all patient sample wells in which toxin additions were made. Dispel pipette tips between each set.

11.1.7 Incubate the U-bottom plate at 37°C for 15 minutes.

11.1.8 During incubation, prepare two new Costar #3799 U-bottom plates with 200 uL of room temperature SP2/Tac cell media in all used rows.

11.1.9 Add 10 uL of .2% PBS/HSA to row C of U-bottom plate #2.

11.1.10 When the 15 minute incubation of plate one is complete, add from plate one to plate two in the following manner:

Using a single channel pipette:
Transfer 10 ul from plate 1, well A1 to plate 2, wells A1-A6.
Transfer 10 ul from plate 1, well A2 to plate 2, wells A7-A12.
Transfer 10 ul from plate 1, well A3 to plate 2, wells B1-B6.
Transfer 10 ul from plate 1, well A4 to plate 2, wells B7-B12.

Using a multichannel pipette:

Transfer, in triplicate, 10 ul from patients wells of plate 1 into the corresponding wells of plate two. For example, patient #1 sample from well D1 of plate one is transferred to wells D1-D3 of plate two. Patient #1 sample from well D2 is added to wells D4-D6 of plate two, etc. Continue to add samples in this manner until all Patient samples have been added to plate 2.

11.1.11 After transfers are complete, mix all wells of plate two using a multichannel pipette. Be sure to dispense tips between rows.

11.1.12 Using a 12-tip multichannel pipette, make direct transfers of 18.5 ul from plate 2 into plate 3 by row. For example, transfer wells A1-A12 of plate 2 into wells A1-A12 of plate 3. Mix plate 3 using a multichannel pipette as explained previously.

11.1.13 As in step 12, make a direct transfer of 50 ul from plate 3 into the cell plates prepared before start of assay. Gently add dilution to the cell plate (do not pipette up and down).

11.1.14 Incubate the SP2/Tac assay plates 18-20 hours at 37°C. Pulse the cell plates using leucine-free RPMI media and 3H leucine at a dilution of 1:50 (1 uCi/well). Gently add 50 ul of the 1:50 {^{125}I}-leucine dilution to the assay plates using a multichannel pipette. Dispense tips between rows. Incubate the plates for 41/2 hours at 37°C.

11.1.15 The cell plates are harvested after incubation using a MACH III Tomtek cell harvester. Harvester instructions located in protocol book in room 428A. The cells are harvested onto filter mats and read using a Wallac Beta Counter and the data is electronically captured.

Reasons to repeat an assay:
1. Failure to get counts above 2500.
2. Failure of the standard curve.
3. Low counts in serum only tube (as compared to subsequent serum dilutions).
4. PI requests a repeat of assay.

11.2 BIOASSAY FOR BLOOD LEVELS OF LMB-2

11.2.1 Plate SP2/Tac cells at 40,000/well in flat bottom plates. A typical PK assay requires 3 plates (I, II, and III).

11.2.2 Label 2 U-bottom plates (I & II) and add room temperature SP2/Tac media in the following fashion:

a. In U-bottom plate I: wells A1-A5 receive 200 ul, wells A6-A7 receive 198 ul/well, A8 receives 205 ul, columns 1-3 starting with row B receive 198 ul, and column 4 receives 205 ul starting with row B.

b. In U-bottom plate II: wells A1-4 and A7-10 receive 200 ul of media, columns 1-3 and 7-9 starting with row B receive 198 ul, and columns 4 and 10 starting with row B receive 205 ul.

11.2.3 Add 8.5 ul of each plasma time point to column 4 starting with row B of U-bottom plate I in sequential order. It is very important to double-check the order of the time points. Day 1 time points are added first in order of time drawn, then day 3, etc. A total of seven time points can be added to U-bottom plate I.

11.2.4 Proceed to U-bottom plate II. Additional time points, in sequential order, can be added first to column 4 starting with row B, then to column 10 also starting with row B. Row A is left blank in both U-bottom plates as Row A is used for the titration row. Dr. Kreitman HSA requested a total of two "blank" rows per PK assay. These rows receive only media in the final step of the assay (no toxin or plasma is added to these rows). The assay must be structured so that a total of two rows in the flat bottom plates are not used. A total of 19 time points can be run on three plates. If more than 19 time points are to be run, it will be necessary to use an additional cell plate IV.

11.2.5 After the plasma samples are added to the U-bottom plates, an 8 well multichannel pipette is used to the dilutions. The same procedure is used on each U-bottom plate starting with row B. Transfer 22 ul from column 4 into column 3 (i.e. 22 ul from B4 is transferred into B3, etc.). Column 3 is mixed well and 22 ul is transferred from column 3 into column 2. Finally, 22 ul is transferred from column 2 into column 1. The same procedure is used to make dilutions from column 10 through column 7 of U-bottom plate II. It is important that thorough mixing is performed after each dilution and before the next transfer is made.

11.2.6 Add 8.5 ul of LMB-2 toxin to well A8 of U-bottom plate I. Mix well. Transfer 22 ul of A8 into A7, 22 ul of A7 into A6, 50 ul of A6 into A5, 50 ul of A5 into A4, 50 ul of A4 into A3, 50 ul of A3 into A2, and 50 ul of A2 into A1. Again, thorough mixing is necessary in each well before each transfer is made.

11.2.7 Transfer 50 ul from well A5 of U-bottom plate I into wells A4 and A10 of U-bottom plate II. A series of 50 ul transfers are made from A4 to A3, from A3 to A2, and from A2 to A1 with thorough mixing after transfer. Next, 50 ul transfers are made from A10 to A9, from A9 to A8, and from A8 to A7. This is to assure a titration curve for each of the three cell plates of the PK assay. Once the toxin is added to the U-bottom plates and mixed, the final transfer to the cell plate is made. At this point, there should be three sets of four columns containing serum and toxin dilutions on the U-bottom plates I and II.

11.2.8 The PK assay does not require a 15 minute incubation before addition of samples to the cell plates. Therefore, a multichannel pipette is used to transfer a 50 ul aliquot from each column of the U-bottom plates to the SP2/Tac cell plates in triplicate. For example, 50 ul from column 1 of U-bottom plate I is added to columns 1-3 of SP2/Tac cell plate I, column 2 of U-bottom plate I is added to 4-6 of cell plate I, column 3 to columns 7-9, and column 4 to columns 10-12 etc.

11.2.9 Similarly, 50 ul from columns 1-4 of U-bottom plate II are transferred to SP2/Tac cell plate II in triplicate and columns 7-10 of U-bottom plate II are added to SP2/Tac cell plate III in triplicate. Remember to add 50 ul of media (without toxin or plasma) to the

rows used as blank rows. If media is not added to these rows, the counts will be slightly higher and will not accurately represent the control value.

11.2.10 Incubate the plates for 18-20 hours at 37°C. End of DAY 1.

11.2.11 DAY 2

Following overnight incubation at 37°C, the plates are pulsed, harvested, and counted as in the neutralization assay.

Reasons to repeat assay:

1. Failure to get counts above 2500.
2. Failure of standard curve.
3. Draw points run in improper order.
4. Repeat requested by Dr. Kreitman.

11.2.12 Follow Up

For follow-up studies in patients off-treatment, which will not affect eligibility for enrollment or retreatment, a non-radioactive neutralization assay may be used, under non-CLIA conditions. For additional correlation between the 2 assays, serum samples are saved to enable the new assay to be run with historical samples previously tested using the radioactive CLIA assay..

12 STUDY CALENDAR

Procedures	Eligibility Evaluation	Every Treatment Cycle											Post-Treatment	
	Prior to Cycle 1	Pre-cycle	Day1	Day 2	Day 3	Day 4	Day 5	Day 6	Days 7-8	Week 2	Week 3	Week 4	8 weeks after CR[6]	Every 3-12 months[5]
Blood sample for CD25 analysis (by FACS)	X	X												X
History (include PS) & physical exam	X	X												
Echocardiogram, stress test	X													
ECG, CXR	X	X												
CT scan of chest/abdomen/pelvis or abdominal MRI[1]	X												X	X
Serum anti-LMB-2 antibody assay, HIV, Hep BsAg & C	X													
Pregnancy test if applicable	X													
Bone Marrow Biopsy	X												X	
Drug administration (LMB-2)			X		X		X							
CBC/diff	X	X			X		X			X	X			X
Acute care panel, hepatic panel, mineral panel	X[3]	X			X		X			X[3]	X[3]			
Total protein	X													
LDH, PT, Beta-2 microglobulin (B2M), SPEP, urinalysis	X													
LDH, urinalysis, uric acid, CK		X			X		X							
Fibrinogen, D-dimer, uric acid, amylase, lipase, IgG, IgA, IgM, CRP, ferritin, haptoglobin, B2M and SPEP		X												
PT, PTT		X												
24 hour Urine for CrCl	X	X												
Pharmacokinetic Studies[2]			X		X		X							
Neutralizing antibodies and soluble Tac		X								X[4]				X[7]

[1] A CT scan (in patients with an abnormal initial scan) will be done routinely after even cycle numbers (2, 4, 6) and will be done at other times if there is clinical evidence that the patient has had a PR, CR, or progressive disease. Ultrasound can replace CT/MRI in the absence of lymph nodes, at the discretion of the PI.
[2] Refer to Section **4.3** for PK collection instructions and schedule.
[3] Albumin is collected instead of mineral panel.
[4] Between Days 17-25.
[5] Refer to Section **4.10**.
[6] Refer to Section **4.4.3**.
[7] Only for patients with PD

13 MEASUREMENT OF EFFECT

13.1 RESPONSE CRITERIA

13.1.1 Complete remission (CR) requires all of the following:

- No evidence of leukemic cells by routine H/E stains of the peripheral blood and bone marrow. Minimal Residual Disease (MRD): CR with HCL evident in blood by flow cytometry (FACS) or in bone marrow by immunohistochemistry. One negative bone marrow biopsy is sufficient to confirm CR providing it is done after the normal blood counts qualify for at least 4 weeks.
- No hepatomegaly, splenomegaly, or lymphadenopathy (not >2 cm in short axis) by physical examination and appropriate radiographic techniques. Adenopathy which persisted but became negative by biopsy would be consistent with CR.
- Normal CBC as exhibited by: Neutrophils ≥ 1,500/uL, Platelets ≥ 100,000/ul, and Hemoglobin ≥ 11.0 g/dL without transfusions or growth factors for at least 4 weeks. After this 4 week period the bone marrow biopsy and CT must be done to confirm CR.

13.1.2 Partial Response (PR) requires all of the following for a period of at least 4 weeks:

- The patient must have the following (if abnormal prior to treatment):
  - ≥ 50% decrease in hairy cell count from the pretreatment baseline value by flow cytometry. Flow cytometry will not prevent consideration of PR or determine relapse from PR if the HCL count by flow cytometry remains below 50 cells/mm^3 and the patient qualifies for PR by other parameters.
  - ≥ 50% reduction in abnormal hepatosplenomegaly by CT or physical exam.
  - No growth in lymphadenopathy. As HCL is not PET-avid, adenopathy which is stable is considered not measureable to determine PR, and would not contradict a PR proven by other criteria.
- and the following:
  - Neutrophils ≥ 1,500/uL or 50% improvement over baseline without growth factors for at least 4 weeks,
  - Platelets ≥ 100,000/uL or 50% improvement over baseline, &
  - Hemoglobin ≥ 11.0 g/dL or 50% improvement over baseline without transfusions or growth factors for at least 4 weeks. The baseline Hgb for patients who are transfusion dependent for red cells is assumed to be 6 g/dl.

13.1.3 Progressive disease (PD): Defined by at least one of the following compared to pretreatment:

- ≥ 25% increase in the sum of the products of the greatest perpendicular dimensions of at least two lymph nodes on two consecutive examinations at least

2 weeks apart (at least one node must be ≥ 2 cm) or appearance of new palpable lymph nodes,

- ≥ 25% increase in the size of the liver and/or spleen as determined by measurement below the respective costal margin, or appearance of new palpable hepatomegaly or splenomegaly that was not previously present,
- ≥ 50% increase in the number of circulating HCL cells by flow cytometry, and >50 cells/mm^3.

13.1.4 Stable disease: (SD) will be characterized by not meeting any of the criteria outlined above.

13.2 MEASUREMENT OF RESPONSE

Assessment of clinical response will be made according to the guidelines as outlined above. The objective measures of response, such as bone marrow slides and CT scans, will be saved so that responses may be audited. Staging will include a CBC/differential, an interim history and physical examination with documentation of measurable disease, and performance status. Physical examination should record the diameter, in two planes, of the largest palpable nodes in each of the following sites: cervical, axillary, supraclavicular, inguinal, and femoral. Physical examination if relevant can record liver and spleen size as determined by measurement below the respective costal margin. A CT/MRI or U/S (in patients with an abnormal initial scan) will be done routinely after even cycle numbers (2, 4, 6, 8, 10) and will be done at other times if there is clinical evidence that the patient has had a PR, CR, or progressive disease.

13.3 CONFIRMATION OF RESPONSE & DURATION OF RESPONSE

13.3.1 Confirmation

The beginning of PR or CR will be considered the earliest time point where all relevant tests are consistent with CR or PR, respectively. CR or PR must be confirmed for at least 4 weeks according to the criteria above. For consideration of CR or PR duration based on the CBC, single unacceptable values preceded and followed by acceptable values, or values up to day 12 of each cycle may be ignored.

13.3.2 Duration of Response

In patients in CR, the bone marrow biopsy and CT scan should be done at least 4 weeks after the last dose of the last cycle, at 6 month intervals for 2 years after CR, and then at 1 year intervals, and if positive would indicate relapse rather than invalidation of the CR. The duration of PR or CR is measured from the date a confirmed CR or PR begins, until the date that staging studies are no longer consistent with CR or PR. The duration of overall response will be measured from the date that a confirmed response begins, until the date that recurrent or progressive disease is objectively documented.

14 DATA REPORTING / REGULATORY CONSIDERATIONS

Adverse event lists, guidelines, and instructions for AE reporting can be found in Section 7 (Adverse Events: List and Reporting Requirements).

14.1 PROCEDURES

This study will be monitored by Clinical Data Update System (CDUS) version 1.X. Cumulative CDUS data will submitted quarterly to CTEP by electronic means. Reports are due January 31, April 30, July 31, and October 31.
For data safety and monitoring, all adverse events will be reviewed by the Principal Investigator and the research team. The data manager and research nurses will be responsible for data entry and reporting. Unexpected adverse events and/or serious adverse events will be reported to the NCI IRB and the study sponsor as described in section 7. If trends are noted and/or risks warrant it, accrual will be interrupted and/or the protocol and/or consent document will be amended accordingly.

14.2 STUDY SPONSOR: CTEP

15 STATISTICAL CONSIDERATIONS

15.1 STUDY DESIGN/ENDPOINTS

This is a nonrandomized phase II study. The primary objective is to 1) determine the response rate of LMB-2 in patients with CD25-positive Hairy Cell Leukemia (HCL).

15.2 SAMPLE SIZE/ACCRUAL RATE

The primary objective of this trial will be to determine if LMB-2 can produce responses in a reasonable proportion of patients at the current dose and schedule and thus warrant further testing. Considering that patients have not been able to to respond well to either cladribine or BL22, an overall response rate of 50% would be reasonable, and < 20% would be considered too low. The trial will be conducted using a Simon two-stage optimal design [75]. With alpha=0.10 (the probability of incorrectly accepting a poor agent), and beta=0.10 (the probability of incorrectly rejecting a good agent), we will try to rule out an undesirably low response probability of 20% (p0=0.20) in favor of a level indicative of acceptable activity, 50% (p1=0.50).

Ten evaluable patients will initially be enrolled. If 0-2/10 demonstrate a CR/PR, then accrual will stop and the agent will be considered inactive. If 3+/10 patients have a CR/PR, accrual will continue until a total of 17 patients have been enrolled. If 3-5 of 17 have a CR/PR, this will be considered insufficient activity, and the agent will not be considered worthy of further development. If 6+/17 have a CR/PR, then the agent will be considered active (and at least potentially able to produce a CR/PR level consistent with 50%). In addition, if there are 6+/17 responding patients, up to 8 additional patients overall (maximum 25 evaluable patients overall) will be allowed to be entered into this trial to be enrolled to provide additional data for secondary endpoints. Confidence intervals will be constructed about the observed response proportions based on a two-stage approach [76]. Under the null hypothesis (p0=0.20), the probability of early termination of this trial is 0.68.

Duration of response is also important to evaluate in this population. A statistical summary of the duration of response will be reported, along with the fraction of responding patients who achieve a six month response duration, and the corresponding 95% confidence interval. In addition, a Kaplan-Meier curve of response duration will be created to illustrate the pattern associated with this outcome.

It is expected that 5-10 patients per year can be recruited for enrollment onto this trial. With a goal of 25 evaluable patients, it is expected that 4 years is a reasonable time frame in which to accrue all needed subjects. The accrual ceiling will be 55 to account for inevaluable participants and screen failures..

Due to LMB-2 supply, no more than a total of 13 patients may be accrued to the protocol until a new clinical lot is ready for use in patients.

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